

October 31, 2003

TO: Adult Program, Center Directors, and Affiliate Program Directors

FROM: Bruce C. Marshall, M.D.
Director of Clinical Affairs

RE: ABPA Consensus Conference Document

The Cystic Fibrosis Foundation is pleased to announce the publication of the proceedings from its June 2001 consensus conference "Recommendations on Diagnosis and Treatment of Allergic Bronchopulmonary Aspergillosis in Cystic Fibrosis" in the 2003:37 (Suppl 3) issue of *Clinical Infectious Diseases*

You can find the complete document on Port CF (www.portcf.org), under Resources and in the "Consensus & Guidelines" folder. Remember, to access this document, you need to have permission as a "Resource Reader" on Port CF. If you do not have access, contact your center's administrator or registry coordinator for assistance. Hard copies of this document will not be distributed.

The CF Foundation recommends that hard copies be printed and placed in the *Clinical Practice Guidelines for Cystic Fibrosis* and in the *Consensus Conference: Concepts in Care* notebook. The CF Foundation also suggests providing hard or electronic copies of this document to other practitioners in your facility.

The CF Foundation gratefully acknowledges the ABPA conference chairs, Richard B. Moss, M.D. and David A. Stevens, M.D., and all of the participants. A list of participants of the ABPA consensus conference can be found on pages 32-33 of this document.

If you have any questions, please contact Leslie Hazle at (800) FIGHT CF or through e-mail at lhazle@cff.org.

Allergic Bronchopulmonary Aspergillosis in Cystic Fibrosis—State of the Art: Cystic Fibrosis Foundation Consensus Conference

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Because of the difficulties of recognizing allergic bronchopulmonary aspergillosis (ABPA) in the context of cystic fibrosis (because of overlapping clinical, radiographic, microbiologic, and immunologic features), advances in our understanding of the pathogenesis of allergic aspergillosis, new possibilities in therapy, and the need for agreed-upon definitions, an international consensus conference was convened. Areas addressed included fungal biology, immunopathogenesis, insights from animal models, diagnostic criteria, epidemiology, the use of new immunologic and genetic techniques in diagnosis, imaging modalities, pharmacology, and treatment approaches. Evidence from the existing literature was graded, and the consensus views were synthesized into this document and recirculated for affirmation. Virulence factors in *Aspergillus* that could aggravate these diseases, and particularly immunogenetic factors that could predispose persons to ABPA, were identified. New information has come from transgenic animals and recombinant fungal and host molecules. Diagnostic criteria that could provide a framework for monitoring were adopted, and helpful imaging features were identified. New possibilities in therapy produced plans for managing diverse clinical presentations.

OVERVIEW OF ASPERGILLUS-HUMAN ENCOUNTERS AND ALLERGIC BRONCHOPULMONARY ASPERGILLOSIS (ABPA)

Aspergillus fumigatus, a widely distributed spore-bearing fungus, causes multiple diseases in humans [1–3]. These diseases include invasive pulmonary aspergillosis,

aspergilloma, and different forms of hypersensitivity diseases. Pneumonia due to *Aspergillus* and systemic aspergillosis occur primarily in patients who have immunosuppression or T cell or phagocytic impairment. The immunodeficiency detected in these patients may be congenital, acquired, or iatrogenic. Patients with chronic granulomatous diseases, neutropenia, or neu-

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Clinical Infectious Diseases 2003;37(Suppl 3):S225–64

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1058-4838/2003/3707S3-0004\$15.00

The Consensus Conference took place 12–13 June 2001 in Bethesda, Maryland. The convenors were R.B.M. and D.A.S.

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trophil dysfunction and patients with severe immunodeficiency are at risk for the development of this predominantly fatal infection. Although no important protective antibody response was detected, a CD4⁺ Th1 cytokine pattern was suggested to be important in rendering protection in these patients [4].

Hypersensitivity lung diseases include allergic asthma, hypersensitivity pneumonitis, and ABPA; all result from the exposure to allergens of *A. fumigatus*. *Aspergillus* spores on inhalation trigger an IgE-mediated allergic inflammatory response in the bronchial airways, leading to bronchial obstruction and asthma [5]. The immune response to *Aspergillus* antigens in these asthmatic patients is characterized by a Th2 response [6, 7]. Hypersensitivity pneumonitis is characterized by dyspnea due to pulmonary restriction and “influenza-like” syndrome due to fever and fatigue [5]. Serum IgE titers are usually very low in hypersensitivity pneumonitis, and eosinophilia is often insignificant. During the acute phase of hypersensitivity pneumonitis, infiltration of neutrophils has been detected, whereas, during the chronic phase, the inflammatory cells are represented predominantly by T cells and macrophages. This disease is the result of a predominant Th1 type of response, in contrast to other allergic diseases caused by *A. fumigatus* [5].

ABPA develops from sensitization to allergens from *A. fumigatus* present in the environment. Development of allergy to *A. fumigatus* depends on the mode and frequency of exposure. Sensitization to *A. fumigatus* allergens usually occurs in combination with other aeroallergens. In atopic persons, exposure to fungal spores and hyphal fragments leads to the production of specific IgE [1, 8–10].

ABPA is a disease primarily occurring in patients with asthma (1%–2% of asthma patients) or with cystic fibrosis (CF; 1%–15% of CF patients) [7, 10–40]. This disease is characterized by a variety of clinical and immunologic responses to antigens of *A. fumigatus*, which colonizes the bronchial trees of patients. It is manifested by wheezing, pulmonary infiltrates, and bronchiectasis and fibrosis. Some immunologic manifestations are peripheral blood eosinophilia, immediate cutaneous reactivity to *A. fumigatus* antigen, elevated total levels of serum IgE, presence of precipitating antibody to *A. fumigatus*, elevated specific serum IgE and IgG antibodies to *A. fumigatus*, and increased serum concentrations of IL-2 receptor (IL-2R) [41, 42]. The hyphae of *A. fumigatus* that grow saprophytically in the bronchial lumen result in persistent bronchial inflammation, leading to bronchiectasis in patients with asthma. The bronchiectasis is frequently central (proximal), in the central lung field (inner two-thirds), on CT examinations.

BIOLOGY OF ASPERGILLUS

Aspergillus occupies its own genus, which is closely related to *Penicillium* in the fungal kingdom. *Aspergillus* are ascomycetes

and are classified in the form subdivision Deuteromycotina, because most species do not have a sexual reproductive cycle. The most common species of *Aspergillus* causing invasive disease include *A. fumigatus* (90% in some series), *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus terreus*, and *Aspergillus nidulans*, and the most common allergens include *A. fumigatus* and *Aspergillus clavatus*. *Aspergillus* is the only organism that regularly produces both invasive, life-threatening disease and allergic disease in humans [43].

Until recently, which species of *Aspergillus* is causing disease has not been thought to be therapeutically important. Now, intrinsic resistance to amphotericin B has been noted in *A. terreus* [44–46]. Resistance to amphotericin B has also been described in *A. fumigatus* and *A. flavus* [47]. Itraconazole resistance has been noted in *A. fumigatus* but not other species [47]. Of importance for allergic disease, serologic tests have not been extensively studied for *Aspergillus* species other than *A. fumigatus*.

Pathogenic *Aspergillus* generally grow easily and relatively quickly on routine bacteriologic and mycological media in the clinical laboratory. Only pathogenic species are capable of growth at 35°C–37°C [48], and *A. fumigatus* in particular is capable of growth at ≥50°C. *Pseudomonas aeruginosa* may inhibit the growth of *Aspergillus*. Recent comparative work from a large retrospective series established that a higher yield was obtained with use of mycological media than with standard bacteriologic media in the clinical setting [49], and this is recommended whenever a fungal infection including *Aspergillus* is considered. Most isolates of *A. fumigatus* are capable of growth at oxygen tensions as low as 0.1% oxygen [50] but rarely grow on anaerobic plates.

It takes ~12–14 h for *A. fumigatus* to germinate at 37°C on defined simple media but only 4–5 h on rich media. Before germination, conidia swell to ~4–8 times their original volume, and their hydrophobic protein coat is replaced by another cell wall exterior. Later, hyphae appear and logarithmic-phase growth commences. In vitro, hyphal extension and overall fungal mass increases logarithmically until ~24 h, when the growth rate starts to plateau [51]. Branching of hyphae occurs early. *A. fumigatus* has a doubling time of 48 min and a hyphal extension rate of 1–2 cm/h when grown with hydrocortisone [51]. Hydrocortisone accelerates the linear (specific) growth rate by 30%–40% in *A. fumigatus* and *A. flavus* and cell wall synthesis by >150%. There are also discernible differences in growth rate between different species of *Aspergillus*, with the most rapidly growing organism being *A. fumigatus*, and probably differences between isolates also exist.

Growth rate at 37°C may be one determinant of the rate of progression of disease and possibly pathogenicity. In addition, *A. fumigatus* has some other characteristics that may contribute to pathogenicity. These include very small spore size (3–5 μm),

which enables the spores to penetrate deeply into the lung. Spores are capable of withstanding extraordinary atmospheric conditions (and suboptimal host defenses), probably by virtue of the hydrophobic protein coat layer composed of rodlet fascicles [52–54]. The pigment of conidia also confers some protection against phagocytosis [55]. The first immunologic line of defense against *Aspergillus* in the lungs, and presumably the nose, is the macrophage, which is capable of ingesting and killing spores [56]. Both monocyte-derived and resident macrophages contribute to spore ingestion and killing. Killing is done by an opsonin-independent nonoxidative method. This line of defense is depressed by glucocorticoids, not via depression of T cells but possibly via failure of phagolysosomal fusion plus an effect on cytokine production. Granulocyte-macrophage colony-stimulating factor (GM-CSF) reverses the steroid depression [57–59].

Despite the hydrophobic exterior, *A. fumigatus* conidia bind surfactants A and D; various extracellular matrix proteins, such as laminin, fibronectin, and fibrinogen; and mannose-binding lectin efficiently, as well as C3 [60–64]. Surfactant enhances phagocytosis of conidia. Several human polymorphisms of surfactant proteins with functional consequences have been described [65, 66], as have polymorphisms of mannose-binding lectin [67], leading to the speculation that differences in the manifestations of aspergillosis may relate in part to host differences in interacting with *Aspergillus* conidia and hyphae.

The second line of defense, once conidia have germinated, is neutrophil and monocyte killing of hyphae. This can occur even in the absence of opsonins or activation. Both oxidative mechanisms and defensins appear to be involved. Monocytes principally use hydrogen peroxide. Steroids suppress this line of defense, probably in part by their suppression of the oxidative burst and possibly by decreasing cellular mobilization.

Aspergillus produces a number of superoxide dismutases [68], at least 2 catalases [69–71], and mannitol [72, 73]. These may protect the organism from damage from singlet oxygen, hydrogen peroxide, hydroxyl, and other free radicals produced by phagocytes. One superoxide dismutase (Asp f6) and catalase B are immunogenic.

Various putative virulence determinants of *A. fumigatus* have been described. Conidia produce an inhibitor of the oxidative burst and of proinflammatory cytokine production. The fungus also produces various proteases [74–76], ribotoxin [77, 78], phospholipases [79], a hemolysin [80], gliotoxin [81], phthioic acid [82], and other toxins [83]. Work with the alkaline protease of *A. fumigatus* with use of both single or double deletants in carefully controlled animal model experiments has failed to show the importance of alkaline protease in invasive aspergillosis [84]. However, one protease was able to induce pulmonary epithelial cell detachment, as well as inducing proinflammatory cytokine release [85]. Several proteases are immunogenic, in-

cluding Asp f5, f10, f13, f15, and f18. Similarly, ribotoxin (Asp f1) does not appear to be important in the pathogenesis of invasive aspergillosis [86]. Gliotoxin has been shown to reduce macrophage and neutrophil phagocytosis [81]. Phthioic acid may contribute to granuloma formation, as it may do in tuberculosis. A histidine kinase has been reported to be a virulence factor [87].

IMMUNOPATHOGENESIS OF ABPA IN CF

The immune response to *Aspergillus* antigens in patients with ABPA, as well as in allergic asthmatic patients and patients with CF, is a Th2 CD4⁺ cell response [7]. A central question, then, is how ABPA differs from *Aspergillus* sensitivity in atopic asthma and CF. It is proposed that ABPA develops in genetically susceptible asthmatic patients and patients with CF because of increased frequency and/or activity of *A. fumigatus*-specific Th2 CD4⁺ cells.

The allergic inflammatory response in patients with ABPA appears to be quantitatively greater than that in *Aspergillus*-sensitive atopic asthma patients and patients with CF. In the proposed model of the immunopathogenesis of ABPA, as illustrated in figure 1, *A. fumigatus* spores are inhaled into the bronchial airway, where they are trapped by the luminal mucus, germinate, and form mycelia. *A. fumigatus* mycelia release allergens that are processed by antigen-presenting cells bearing HLA-DR2 or -DR5 and presented to T cells within the bronchoalveolar lymphoid tissue (BALT). The T cell response to *Aspergillus* allergens becomes skewed toward a Th2 CD4⁺ cell response, with synthesis and secretion of cytokines IL-4, IL-5, and IL-13.

***Aspergillus* antigens and bronchial epithelia.** One of the characteristic features in patients with ABPA is that *A. fumigatus* is found bound to the surface epithelium and is growing on and between the epithelial cells without being efficiently killed by mononuclear and eosinophilic infiltrates [88]. It has also been shown that spores of *A. fumigatus* are attached to epithelial surfaces cultured in vitro [89]. The physical presence of *A. fumigatus* on and between the epithelial cells is possibly of importance for the modulation of the immunologic response toward a Th2-type response [90]. Over the past decades, virulence factors of *A. fumigatus* that interfere with or even block normal functions of the humoral and cellular defense of the airways have been detected [91, 92]. Virulence factors were discussed above. Some of these virulence factors are the proteolytic enzymes of *A. fumigatus*.

Certain strains of *A. fumigatus* have been found to release proteolytic enzymes with elastolytic and collagenolytic activities. The possible role of these proteolytic enzymes as a pathogenic factor in fatal invasive aspergillosis is still uncertain. However, findings in patients with ABPA or aspergilloma in-

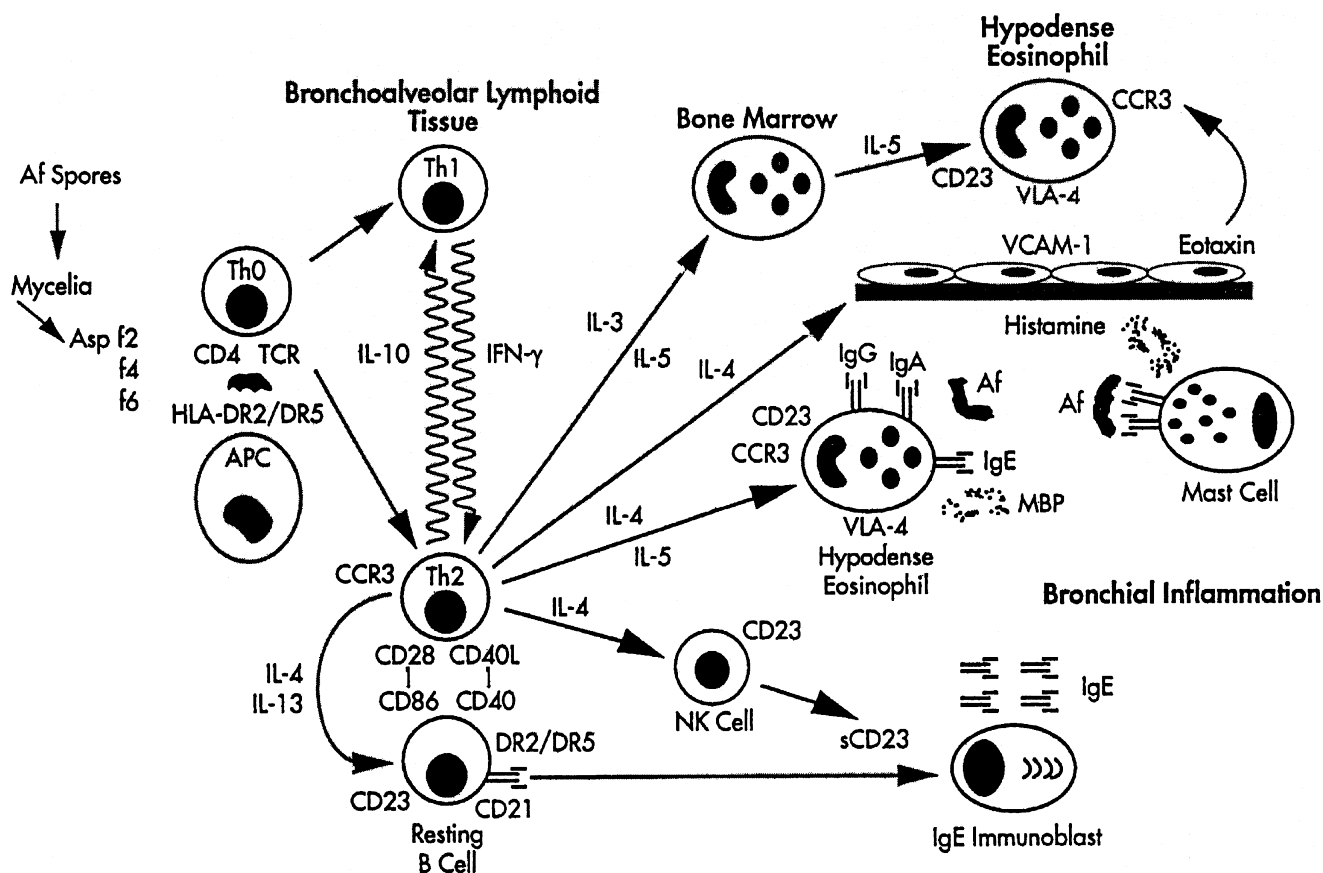


Figure 1. Model of pathogenesis of allergic bronchopulmonary aspergillosis (from [7]). Af, *Aspergillus fumigatus*; APC, antigen-presenting cell; CCT3, CC chemokine receptor 3; L, ligand; MBP, major basic protein; NK, natural killer; s, soluble; TCR, T cell receptor; VCAM, vascular cell adhesion molecule; VLA, very late antigen.

dicates that these proteases may be involved in the pathogenesis of these diseases. In recent studies, culture filtrate extracts with marked elastase and collagenase activity were used in Western blotting experiments to show binding of IgG antibodies to a 32-kDa elastase protein in serum samples from patients with ABPA or aspergilloma [93]. The pronounced binding of IgG antibodies with the 32-kDa fungal elastase suggests that these proteases are produced *in vivo* in patients with ABPA and aspergilloma. Furthermore, it was found that, during exacerbations of aspergilloma, concentrations of antibody to different antigens, including the 32-kDa and 40-kDa proteases, were markedly increased [94]. The observation that concentrations of antibody to the 32-kDa and 40-kDa fungal proteases are increased during the exacerbation phase indicates that these proteases may play a role in the pathogenicity of the disease.

An important feature of pathogenic microbes is their capacity to interact with epithelial cells of the mucosal surface. It has been shown elsewhere that products released *in vitro* by *A. fumigatus* are able to cause epithelial cell detachment [85, 95]. This capacity to induce epithelial cell detachment is also characteristic of other proteases released by different fungi (e.g.,

Alternaria and *Cladosporium*), but *Aspergillus* proteases were more active at much lower concentrations [96]. Recent studies with different proteases from various sources, for example, Der p1 from the house dust mite [97], have shown that degradation of epithelial cell structures will result in facilitated transport of antigens and allergens across the epithelial cells, resulting in enhanced exposure to antigen-presenting cells and concurrent immune responses.

In addition to damaging the integrity of the epithelial cell layer, recent studies have shown that human bronchial and alveolar epithelial cell lines produced proinflammatory cytokines, such as IL-8, IL-6, and monocyte chemoattractant protein 1 (MCP-1), after incubation with protease-containing culture filtrates of *A. fumigatus*. This cytokine-releasing activity could be ascribed to the proteolytic activities of these extracts [85]. These observations suggest that proteolytic enzymes released by *Aspergillus* growing on and between epithelial cells may be responsible for the induction of chemoattractive cytokines by epithelial cells and corresponding inflammatory responses. It has been proposed that induction of a severe inflammatory response by the direct activation of epithelial cells

may induce additional harm to the epithelial cell layer [91]. Destruction of the epithelial cell barrier either by proteases from the fungus or by eosinophilic and neutrophilic inflammation is followed by repair mechanisms, resulting in the influx of serum proteins and extracellular matrix proteins to the lumen site of the epithelium [98]. Because spores and mycelium of *A. fumigatus* have surface structures that are able to interact with extracellular matrix molecules, damage and concurrent repair mechanisms of the airway mucosa may facilitate the binding of *Aspergillus* to the damaged sites of the airways. The enhanced release of proteolytic enzymes and allergens on the epithelial surface will induce a continuous inflammatory response and mast cell degranulation, resulting in severe and long-lasting periods of exacerbations of ABPA.

In addition to the induction of cytokine responses of epithelial cells, it has been shown that proteases from *A. fumigatus* at higher concentrations also caused hypofunction of epithelial cells, even below the spontaneous cytokine production of epithelial cells; this is in contrast to proteases from other fungi, which do not reduce cytokine production [96]. This silencing mechanism of the epithelial responsiveness, which was specific for the elastase- and collagenase-containing extracts of *A. fumigatus*, may represent an additional virulence factor by preventing effective targeting by infiltrative phagocytic cells, because of the lower concentrations of chemokines in the direct environment of the fungus. This continuous release of antigens and allergens will induce a strong activation of the Th2-type immunologic response, with very high production of total and specific IgE antibody, and an additional Th1 response, with formation of IgG and IgA antibodies to antigens of *A. fumigatus*, as is observed in patients with ABPA.

Th2 cells. Several groups have observed T cell lymphoproliferative responses to crude *Aspergillus* extracts [99–102]. Subsequently, *Aspergillus*-specific T cell responses were examined. In these studies, T cells were stimulated with a crude *Aspergillus* extract for 48 h, and the *Aspergillus*-stimulated T cell supernatant was then cocultured with atopic control B cells for 10 days. These supernatants, obtained from *Aspergillus*-stimulated T cells from patients with ABPA, enhanced B cell IgE synthesis [6]. Subsequently, T cell lines were generated from patients with ABPA by use of an *Aspergillus* allergen, Asp f1 [103]. In these studies, the T cell phenotype was exclusively CD4⁺CD25⁺ HLA-DR⁺. The cytokine profile of these T cells was IL-4⁺, IFN- γ ⁻ (i.e., Th2) CD4⁺ T cells. Indeed, the lymphoproliferative stimulus for Asp f1 T cell lines was predominantly IL-4 mediated: an autocrine pattern inhibited by anti-IL-4 but not anti-IL-2 [103]. However, atopic *Aspergillus*-sensitive patients with CF also developed Th2 CD4⁺ cells. Subsequent studies by Chauhan and colleagues [104, 105] demonstrated that T cell clones obtained from asthmatic patients with ABPA were either Th2 (IL-4⁺, IFN- γ ⁻) or Th0 (IL-4⁺,

IFN- γ ⁺). Furthermore, tetanus toxoid-generated T cell clones showed the expected Th1 phenotype, namely IFN- γ ⁺. Thus, the Th2 CD4⁺ cell response in ABPA is specific to *Aspergillus* antigens and is not a generalized Th2 cell response to all antigens.

IL-4 plays a critical role in the allergic inflammatory response in ABPA (figure 1). IL-4 up-regulates cellular activity via binding to IL-4R found on a variety of cells, including B cells, natural killer (NK) cells, mast cells, endothelial cells, and a subpopulation of T cells [106–111]. IL-4 and IL-13 induce IgE isotype switching of B cells [112–117]. Although IL-4 is necessary for IgE isotype switching, it is not sufficient. For IgE secretion to occur, a second signal mediated by cell-cell T and B cell interactions via CD40L-CD40 and CD28-CD86 ligand-receptor interactions is needed [115–127]. IL-4 also induces the low-affinity IgE receptor CD23 and soluble CD23, which augments B cell IgE synthesis [115, 116, 128–133]. In addition, T cell CD23 and B cell CD21 cognate ligation augments B cell IgE synthesis. CD86 expression on B cells is also up-regulated by IL-4 in atopic patients [118–126]. CD86 on B cells is an important costimulatory molecule for augmentation of IgE synthesis. The ligand for CD86 is CD28 on T cells. In addition, several studies have observed CD86 to be critical in promoting Th2 CD4⁺ cell responses and cytokine synthesis, eosinophil airway inflammation, and airway hyperresponsiveness after allergen challenge. Because ABPA is characterized by a heightened Th2 CD4⁺ cell response to *A. fumigatus* allergens and a hyper-IgE state, it is hypothesized that a reason for this response is increased sensitivity to IL-4 stimulation in ABPA, which results in increased expression of CD23 and CD86, leading to a positive-feedback amplification mechanism that also increases Th2 CD4⁺ cell responses.

In recent studies, it has been observed that B cells from patients with ABPA were significantly more sensitive to IL-4 stimulation than were cells from atopic and nonatopic patients, with up-regulation of CD23 and CD86 expression [134]. At day 0, before culture, the number of CD23 molecules per CD20⁺ B cell was significantly elevated in vivo in patients with ABPA compared with numbers in atopic and nonatopic patients [134]. After in vitro IL-4 stimulation for 48 h, patients with ABPA had significantly increased rates of CD23 expression per B cell compared with rates in atopic and nonatopic subjects. Furthermore, there were significantly increased numbers of CD23⁺ molecules per CD86⁺ B cell after IL-4 stimulation in patients with ABPA compared with numbers in atopic and nonatopic patients [134]. Similarly, both patients with ABPA and atopic patients had increased expression of CD23⁺ and CD23⁺ CD86⁺ B cells at day 0 before culture compared with that in nonatopic patients, again indicating in vivo up-regulation. After 48 h of culture of cells with IL-4, patients with ABPA had significant up-regulation of CD23⁺ CD86⁺ B cells compared with that in atopic and nonatopic patients. When

patients with ABPA and CF were compared with patients with CF but not ABPA, this pattern of increased numbers of CD23⁺ and CD23⁺ CD86⁺ B cells was observed [134]. Thus, patients with ABPA had increased sensitivity to IL-4 stimulation, with up-regulation of CD23 and CD86 expression compared with that in other atopic persons, such that patients with ABPA > atopic patients >> nonatopic patients. It seems that in ABPA there is a positive-feedback amplification loop mechanism of CD86⁺ B cell and Th2 CD4⁺ cell stimulation by IL-4.

The model proposes that *A. fumigatus* growing within the airways releases high levels of allergens in the airway and lung parenchyma, which in turn produces a heightened and prolonged late-phase allergic inflammatory response. Furthermore, patients with ABPA develop IgE antibodies to the specific *Aspergillus* proteins Asp f2, Asp f4, and/or Asp f6, whereas atopic patients develop IgE antibodies to Asp f1 and/or Asp f3 [135–140]. It is hypothesized that mycelial formation and secretion of proteins in ABPA is necessary to trigger these events, suggesting that the colonization in patients with ABPA is greater than that in *Aspergillus*-sensitive atopic patients. This increased exposure to *Aspergillus* allergens occurring in a genetically susceptible host then drives the skewed Th2 CD4⁺ cell and hyper-IgE responses seen in patients with ABPA.

Recently, studies have been initiated to determine whether there is increased frequency of Th2 CD4⁺ cells in patients with ABPA (table 1). The frequency of cytoplasmic IFN- γ ⁺ and IL-4⁺ CD3⁺ T cells in phorbol myristate acetate- and ionomycin-stimulated cultures was comparable in patients with CF with and without ABPA, indicating that there was no skewing of Th2 CD4⁺ cell responses. Interestingly, IFN- γ ⁺ CD3⁺ T cells

were significantly decreased in patients with CF with and without ABPA compared with cell numbers in nonatopic controls. When antigen-specific frequencies of Th1 and Th2 responses were evaluated, a different picture emerged. The frequency of IFN- γ ⁺ CD3⁺ T cells in tetanus toxoid-stimulated cultures was similar among patients with CF with or without ABPA and tended to be decreased compared with that among nonatopic controls. However, in Asp f2-, f3-, and f4-stimulated cultures, the frequency of IL-4⁺ CD3⁺ T cells was significantly increased among patients with CF and ABPA compared with that among patients with CF without ABPA. This suggests that there is an increased frequency of *Aspergillus*-specific Th2 CD4⁺ cells among patients with CF and ABPA compared with that among those without ABPA.

Because the immune response to *Aspergillus* antigens originates in the BAL, investigators have examined bronchoalveolar immunity. The cells obtained from bronchoalveolar lavage (BAL) fluid from patients with ABPA are an admixture of alveolar macrophages, eosinophils, and lymphocytes, similar to those found in samples from individuals with asthma [113, 114, 141, 142]. Eosinophil infiltration predominates in both BAL fluid and lung tissue, as is evident on lung biopsy [88]. In addition, eosinophils are activated and have released their mediators, such as major basic protein. Thus, eosinophils are a major effector cell causing inflammation. Lymphocytes found in BAL fluid are composed of T, B, and NK cells. The T cells are an admixture of CD4⁺ and CD8⁺ T cells, in a ratio of ~2 :1. Interestingly, increased numbers of CD23⁺ NK cells and CD23⁺ CD4⁺ T cells obtained from BAL fluid from patients with ABPA have been observed, indicating in vivo IL-4 stim-

Table 1. Frequencies of Th2 and Th1 CD3⁺ cells in patients with cystic fibrosis (CF) with and without allergic bronchopulmonary aspergillosis (ABPA).

| Stimulant, cytokine | Patients with CF with ABPA | Patients with CF without ABPA | Control subjects | P ^a |
|-------------------------------------|----------------------------|-------------------------------|------------------|----------------|
| Phorbol myristate acetate/ionomycin | | | | |
| No. of patients | 5 | 6 | 3 | |
| IFN- γ | 11.4 \pm 0.09 | 10.5 \pm 1.9 | 31.3 \pm 1.7 | NS, <.01, <.01 |
| IL-4 | 1.5 \pm 0.1 | 1.2 \pm 0.2 | 1.6 \pm 0.2 | ... |
| Tetanus toxoid | | | | |
| No. of patients | 4 | 6 | 2 | |
| IFN- γ | 7.0 \pm 1.8 | 1.9 \pm 0.3 | 11.7 \pm 2.0 | ... |
| IL-4 | 0.4 \pm 0.2 | 0.6 \pm 0.3 | 0.3 \pm 0.0 | ... |
| Asp f2, f3, f4 | | | | |
| No. of patients | 4 | 6 | 2 | |
| IFN- γ | 7.3 \pm 3.0 | 3.5 \pm 0.7 | 13.9 \pm 3.3 | ... |
| IL-4 | 1.7 \pm 0.1 | 0.7 \pm 0.2 | 0.6 \pm 0.2 | <.01, NS, NS |

NOTE. Data are % of cells secreting cytokine in response to stimulant (mean \pm SE), unless otherwise indicated. From A.P.K., J. Consolino, J. Smick, P. S. Hutcheson, V.P.K. (unpublished data). NS, not significant.

^a Student's *t* test comparing ABPA vs. non-ABPA CF, ABPA-CF vs. control, non-ABPA CF vs. control.

ulation [7]. Recently, in preliminary studies, increased in vivo CD23⁺ expression (~10%) on CD4⁺ T cells in patients with CF and ABPA has been observed. The significance of CD23⁺ T cells is probably T cell CD23 and B cell CD21 T-B ligand-counterligand interaction and augmentation of IgE synthesis. Similarly, CD23⁺ NK cells are an important source of soluble CD23 and/or NK CD23–B cell CD21 interaction, increasing immunoblast IgE secretion.

B cells and IgE anti-*Aspergillus* antibodies. In ABPA, extremely elevated total serum IgE concentrations and elevated levels of IgE anti-*Aspergillus* antibody are manifest. There appear to be quantitative and perhaps qualitative differences in the B cell IgE antibody responses in ABPA compared with those in *Aspergillus*-sensitized atopic patients without ABPA. The heightened total and specific anti-*Aspergillus* IgE antibody responses have been described by several groups [14, 18, 143–147]. In ABPA, there are also increased amounts of IgG and IgA anti-*Aspergillus* antibodies, which reflect the Th2 humoral versus Th1 cellular response to *Aspergillus* antigens in these patients [148–155]. Although other *Aspergillus*-exposed groups develop IgE, IgG, and IgA anti-*Aspergillus* antibodies, there is a quantitative increase in IgE anti-*Aspergillus* antibodies in patients with ABPA. B cells obtained from patients with ABPA spontaneously synthesize increased amounts of IgE in vitro compared with that synthesized by *Aspergillus*-sensitized patients without ABPA, indicating in vivo activation of IgE immunoblasts [6]. In preliminary studies, increased numbers of CD23⁺ CD86⁺ B cells were observed in patients with ABPA, which probably accounts for this observation and for the hyper-IgE state [134]. Furthermore, Greenberger and Patterson [144] demonstrated that specific anti-*A. fumigatus* IgE and IgA antibodies are produced within BALT. In contrast, IgG anti-*Aspergillus* antibodies obtained from BAL fluid were predominantly exudative, derived from the peripheral systemic lymphoid system. Slavin et al. [156] demonstrated lymphoid follicles that stained with anti-IgE in a lung biopsy sample from a patient with ABPA, indicating in vivo IgE-bearing B cells and immunoblasts. Thus, BALT B cells have been driven to IgE immunoblasts. Greenberger and Patterson [144] further demonstrated that the total IgE in the systemic lymphoid tissue constituted only a fraction of the specific anti-*Aspergillus* IgE antibodies. This implies that the CD4⁺ Th2 cells have trafficked to the systemic immune system and have activated other clones of B cells, in addition to those with *Aspergillus* specificity.

IL-4R. As a potential mechanism for increased B cell IgE synthesis and secretion, mutations of IL-4R α chain (IL-4R α) have been evaluated. Mutations or polymorphisms of IL-4R α have been identified in atopic persons with elevated IgE levels [109–111]. These polymorphisms increase IL-4 and IL-4R interactions, resulting in a gain of function of IL-4R α that promotes B cell IgE isotope switching. Subsequently, 7 mutations

have been identified that result in increased IL-4R activity [157, 158]. In addition, increased IL-4 activity would result in increased expression of other receptors, including CD23 and CD86 on B cells, eosinophils, and NK cells; very late antigen (VLA)–4 on eosinophils and T cells; vascular cell adhesion molecule (VCAM) on endothelial cells; and CC chemokine receptor 3 (CCR3) and eotaxin secretion (figure 1). In preliminary studies, homozygous mutations of IL-4R α in 2 of 2 patients with ABPA and heterozygous mutations in 3 of 5 atopic patients and 2 of 5 nonatopic control patients have been reported. However, increased sensitivity to IL-4 stimulation was observed in patients with ABPA and atopic patients who had IL-4R α mutations and wild type. IL-4R is a heterodimer, consisting of IL-4R α and the common γ chain (C γ) [115–117]. IL-13R is also a heterodimer, consisting of IL-4R α and IL-13R α . IL-4 stimulates both IL-4R and IL-13R, whereas both IL-4 and IL-13 stimulate IL-4R α /IL-13R α . IL-13, like IL-4, increases CD23 expression, IgE isotype switching, and IgE synthesis. However, IL-13 does not activate and skew Th2 responses.

There is evidence that IL-4R α and IL-13R α interact with the signal transduction protein Janus kinase 1 (Jak-1), whereas C γ interacts with Jak-3. On IL-4 stimulation, IL-4 α and C γ undergo phosphorylation by Jak-1 and Jak-3. After IL-13 stimulation, the IL-13R α is phosphorylated by Jak-1. Phosphotyrosines in IL-4R α and IL-13R α serve as docking sites for the Src homology type 2 domains of the signal transducer and activator of transcription (STAT6) molecule. STAT6 is subsequently phosphorylated by the Jaks, where it is then released as a dimer and translocates to the nucleus, activating IL-4- and IL-13-responsive transcription. The demonstration of increased CD23 expression with IL-4 stimulation suggests that other mutations or polymorphisms of the IL-4R α , IL-13 α , Jak-1, or STAT6 pathway may exist.

Chemokines and integrins. The effector cells responsible for the allergic inflammatory responses in ABPA are predominantly mast cells and eosinophils. In the model (figure 1), when *Aspergillus* antigens cross-link IgE bound to mast cells, mast cells release a variety of mediators, such as histamine, leukotrienes, and platelet-activating factor, which induce bronchial smooth muscle contraction and vascular permeability [7]. A number of mast cell cytokines, such as leukotriene B4 and platelet-activating factor, are chemoattractants for eosinophils. In addition, chemokines such as eotaxin; regulated on activation, normally T cell–expressed and stimulated cytokine (RANTES); and MCP-3, derived from a variety of cell types such as epithelial and phagocytic cells, induce eosinophil chemotaxis and activation [112–114, 159]. Basophil hyperreactivity with increased histamine release, probably due to IL-4 stimulation, also has been reported in ABPA [160]. Th2 CD4⁺ cells secrete cytokines IL-3 and IL-5, which promote bone marrow maturation of eosinophils and activation of eosinophils [142, 161–

165]. Furthermore, IL-4 induces expression of VCAM-1 on vascular endothelial cells and its ligand VLA-4 on T cells and eosinophils. Recent studies have demonstrated selective expression of the eotaxin receptor, CCR3, on eosinophils, basophils, and Th2 cells, and CCR3 expression is up-regulated by IL-4-polarizing conditions [166, 167]. Thus, both chemotactic and cell surface adhesion molecules promote recruitment of Th2 cells and eosinophils within the allergic inflammatory site [113, 114, 168, 169]. Eosinophils possess Fc receptors for IgE, IgG, and IgA, and IL-4 also induces increased expression of the low-affinity IgE receptor CD23 on eosinophils [142]. In addition, IL-4 and IL-5 induce Fc receptors for IgA [142, 170]. In ABPA, significant amounts of *Aspergillus*-specific IgA antibodies are produced within BALT and are present within the bronchial mucus. Thus, both IgE and IgA anti-*Aspergillus* antibodies bound to their Fc receptors on eosinophils trigger mediator release when they engage allergen [142, 171]. When eosinophil-bound IgE, IgA, and IgG are cross-linked by *Aspergillus* antigens, the eosinophils are triggered to secrete inflammatory mediators, such as major basic protein and eosinophil-derived neurotoxin [142].

T cell receptor (TCR)-V β and HLA-DR restriction. Chauhan et al. [104] investigated whether there is unique TCR recognition (T cell epitopes), TCR-V β restriction, or HLA class II restriction that would promote enhanced Th2 responses. Analysis of T cell epitope mapping has revealed 3 immunodominant regions of the Asp f1 protein in patients with ABPA that is recognized by TCR [104]. Their findings were similar to that found in other allergen models. O'Hehir et al. [172] evaluated T cell responses to purified house dust mite allergens. In their model, T cell clones were generated from atopic and nonatopic persons. Significantly, T cell clones from nonatopic persons proliferated in response to allergen stimulation but did not support IgE synthesis, whereas T cell clones from atopic patients did. Furthermore, TCR epitope mapping studies revealed limited numbers of epitopes reacting with TCR [173, 174], TCR-V β restriction or usage [175, 176], and HLA class II restriction [176]. Four major V β chains, V β 3, 6, 13, and 14, react to Asp f1. This will allow the evaluation of whether mutations in the epitope might alter the T cell cytokine and/or lymphoproliferative responses for potential immunotherapy of ABPA. Recently, Chauhan and colleagues [104, 105] showed HLA-DR2 and -DR5 restriction in patients with ABPA. Furthermore, within HLA-DR2 and HLA-DR5, there are restricted genotypes. In particular, HLA-DRB1*1501 and *1503 were reported to provide high relative risk of development of ABPA. On the other hand, 40%–44% of atopic *Aspergillus*-sensitive persons without ABPA have the HLA-DR2 and/or -DR5 type. Further studies indicated that the presence of HLA-DQ2 (especially DQB1*0201) provided protection from the development of ABPA. These results are similar to those found with

purified house dust mite allergens [177–179]. Thus, certain genotypes of HLA-DR2 and -DR5 may be necessary but not sufficient to cause ABPA. Furthermore, Chauhan et al. [105] demonstrated that Asp f1 allergen has a low affinity of binding to HLA-DR. This is consistent with the Th2 cell response previously reported by others, in that strong HLA-DR-antigen-TCR affinity binding favors a Th1 cellular response, whereas low-affinity binding favors a Th2 humoral response [178–182].

CF transmembrane conductance regulator (CFTR). Because ABPA is found in highest incidence among atopic patients with CF, Miller et al. [183] examined *CFTR* mutations in asthmatic patients with ABPA. Six of 11 patients had mutations of the *CFTR* gene; clearly, there was increased frequency of heterozygous mutations of the *CFTR* gene in these asthmatic patients. It has been hypothesized that in CF, the abnormal mucus promotes the trapping of *Aspergillus* spores within the bronchial airway, permitting and perhaps promoting growth of *Aspergillus* mycelia. The significance of the heterozygous *CFTR* mutation with regard to the properties of mucus of asthmatic patients is unclear. The abnormal mucus may allow increased *Aspergillus* colonization within the bronchial airways of patients with CF and those with asthma and may, in a genetically susceptible person, stimulate a Th2 cell response and subsequent ABPA.

Summary. Quantitative increases in the Th2 CD4⁺ cell responses to *Aspergillus* in both the BALT and systemic immune systems characterize ABPA (table 2). A key element in the immunopathogenesis may be BALT exposure to high levels of *Aspergillus* allergens, perhaps because of abnormal mucus properties resulting from *CFTR* mutations. Proteases of *A. fumigatus* may play a role in facilitation of antigen transport across the epithelial cell layer by damaging the epithelial integrity and by a direct interaction with epithelial cell surface receptors, resulting in production of proinflammatory cytokines and corresponding inflammatory responses. Antigen presentation to T cells is characterized by HLA-DR2 and -DR5 restriction of low-affinity antigen binding. In addition, there is restricted TCR-V β usage. Thus, there is an immunogenetic susceptibility to develop ABPA that resides within the HLA-DR-antigen-TCR signaling of the T cells toward a Th2 CD4⁺ cell response. In addition, there may be increased sensitivity of T cells, B cells, NK cells, and eosinophils to IL-4 stimulation because of mutations of IL-4R α and/or the Jak/STAT pathway genes, accounting for the allergic inflammatory response to *Aspergillus*. This leads to a positive-feedback amplification loop of Th2 CD4⁺ cells→IL-4 synthesis→CD23⁺ CD86⁺ B cells. If the hypothesis is confirmed, it would suggest therapeutic targeting at IL-4, IL-4R, and/or CD86. Thus, these results have significance to the atopic state in general. The results of these studies suggest that the airway changes seen in ABPA are an example of airway remodeling due to allergen-induced allergic inflammation seen in asthmatic patients.

Table 2. Summary of immunopathogenesis of allergic bronchopulmonary aspergillosis (ABPA).

| |
|---|
| ↑ Th2 CD4 ⁺ cell response to <i>Aspergillus fumigatus</i> |
| Bronchoalveolar lymphoid tissue mucosal immune response |
| ↑ IL-4, IL-13, IL-5 |
| ↑ IgE total and anti- <i>Aspergillus</i> antibodies |
| ↑ IgA and IgG anti- <i>Aspergillus</i> antibodies |
| ↑ Eosinophils, both bronchoalveolar lymphoid tissue and peripheral |
| ↑ Very late antigen-4 and vascular cell adhesion molecule integrins |
| Migration of eosinophils and lymphocytes into inflammatory lesions |
| ↑ Th2 <i>A. fumigatus</i> -specific cells |
| HLA-DR2 and -DR5 restriction |
| Genotype restriction (DRB1*1501 and *1503) |
| Low-affinity binding |
| Presence of HLA-DQ2 is protective (DOB1*0201) |
| TCR-Vβ restriction (Vβ 3, 6, 13, 14) |
| ↑ IL-4 activity |
| ↑ IL-4 receptor α homozygous α chain mutations (preliminary data) |
| Mast cell degranulation |
| Basophil hyperreactivity |
| ↑ Soluble CD25 during ABPA flares |
| ↑ T suppressor function |
| Anergy to <i>A. fumigatus</i> by delayed-type hypersensitivity |
| Cystic fibrosis transmembrane conductance regulator gene heterozygous mutations |
| ? Effect on mucus properties |

ANIMAL MODELS OF ABPA

Experimental aspergillosis. The experimental animal model of ABPA serves several purposes. Both common and distinct pathological features occurring in natural and experimental diseases are of great interest, because they serve to identify the key elements in pathogenesis. Experimentally induced diseases can be modeled to aid understanding of various parameters, such as antigen and route of exposure, genetic background, and the role of response modifiers in the disease process. Furthermore, animals with targeted gene deletion or with insertion of transgenes can be studied, and the roles of specific cells, receptors, and mediators in pathogenesis can be more precisely defined. The resulting conclusions can be used to formulate hypotheses, which have to be tested for their application to human disease.

Experimental models have been developed to increase understanding of the pathogenesis of *A. fumigatus*-induced diseases. There are 2 major lines of investigation directed at developing animal models; one is aimed at exploration of infectious aspergillosis and the other focuses on allergic aspergillosis. Experimental pulmonary and invasive aspergillosis is commonly studied in immunosuppressed animals by means of treatment with corticosteroids, irradiation, or cytotoxic drugs. Subsequently, the animals are infected systemically or locally

with *A. fumigatus* spores. Models of invasive aspergillosis have been developed in monkey, rabbit, guinea pig, rat, and mouse [184]. In the murine model, protection depends on the secretion of IFN-γ, IL-12, and TNF-α and on the absence of IL-4-secreting CD4⁺ T cells and of IL-10 [185–187]. Models of allergic aspergillosis, including allergic asthma, rhinitis, and hypersensitivity pneumonitis, have been reported. The present review concentrates mostly on the murine model of allergic aspergillosis, with particular reference to antibody response, airway hyperresponsiveness, lung inflammation, cytokine and chemokine responses, T cell and other immune cell responses, and immunomodulatory treatment, such as peptide and immunostimulatory DNA sequence oligodeoxynucleotide (ISS-ODN) immunotherapy and naked DNA vaccination.

***A. fumigatus* antigens.** Currently, >20 recombinant allergens from *A. fumigatus* have been cloned and expressed. Although recombinant antigens are available in pure form, soluble, crude *A. fumigatus* antigen extract is still most commonly used for the induction of experimental disease by intranasal instillation in animals. Spores or plastic beads coated with crude antigen extract, delivered intranasally, are good inducers of respiratory pathology as well [90, 188–190]. The crude extract is a mixture of culture supernatant and mycelial extract. The use of infectious material, such as spores, has been reported as well

[188, 191, 192]. Most protocols use exposures to *A. fumigatus* antigens over a period of 2–5 weeks; the longest reported experiments span 10–12 weeks [190, 193].

Most of the major antigens associated with ABPA appear to be constituents of the crude extract [194–197]. Interestingly, potent sensitization to the extract occurs in the absence of exogenous adjuvants [194]. The various toxins and enzymes, such as proteases, present in the extract may serve as adjuvants, perhaps by inducing epithelial damage and allowing normally excluded antigens to bypass the mucosal barrier [85, 198–200]. Because proteases have been implicated to preferentially induce Th2 rather than Th1 responses, proteases may be involved in skewing the response to *A. fumigatus* to a more allergic phenotype [85].

Crude extracts can contain toxic molecules, including hemolysin, fumitremorgin, fumigallin, helvolic acid, and gliotoxin [85, 199]. Analysis of the crude extracts by Western blotting with serum from patients with ABPA reveals bands that correspond in size to known antigenic components, including Asp f1, a ribotoxin, proteases, and carboxidases [85, 200]. Thus, the extract is highly antigenic and contains biologically active substances. Extracts containing Asp f1 are highly toxic for mice, as are some of the other toxins.

Antibody response in *A. fumigatus* antigen-sensitized animals. In *Aspergillus*-induced allergy, a strong Th2 response with pronounced antibody production is detected [6–8]. As shown by use of a number of other immunogens, IL-4 is critical for the production of IgE in experimental ABPA. IL-4-deficient (IL-4 $-/-$) mice or mice treated with neutralizing anti-IL-4 antibodies throughout the sensitization period do not have an increased serum IgE response [189, 194, 201]. However, these mice make similar levels of IgG1 antibodies and increased levels of IgG2a antibodies compared with those in sensitized wild type mice [189, 201].

The role of antibodies in the pathogenesis of ABPA was addressed by use of mice with a targeted deletion of the ϵ chain [202]. 129SvEv ϵ chain-deficient mice are not capable of making IgE. C57BL/6 ϵ chain-deficient mice lack not only antibodies of all isotypes but also mature B cells. Sensitization via the intranasal route with *A. fumigatus* antigens induced histological lesions in the lung, which are similar in quality and extent in both types of gene-targeted mice relative to lesions in wild type mice. These lesions typically consist of airway inflammation and perivascular and peribronchial infiltrates [202, 203]. These results are in accord with data obtained with B cell-deficient mice exposed to ovalbumin [204, 205]. Furthermore, prominent alveolar lesions, including granulomas, were induced in response to *A. fumigatus* antigens in the absence of mature B cells and antibodies. Lower numbers of IL-4-producing T cells consistently accumulated in the lungs of sensitized B cell-deficient mice than in lungs of wild type mice

[203]. Both IgE-deficient mice and B cell-deficient mice sensitized with *A. fumigatus* antigens developed airway hyperreactivity to the same extent as did wild type mice [202, 203]. The published data with ovalbumin used as immunogen, in which B cell-deficient mice failed to show airway hyperreactivity, are controversial, although the histological lesions were comparable to those in wild type mice [205, 206]. The eosinophilia was also comparable to that in wild type mice exposed to *A. fumigatus* antigen. In a monkey model of ABPA, allergic human serum or control serum was infused into primed animals. Inhalation of *A. fumigatus* antigens led to the development of severe lung lesions typical of ABPA only in the monkey that had received allergic human serum [207]. However, this monkey failed to demonstrate airway hyperreactivity.

Peripheral blood and lung eosinophils in *Aspergillus*-sensitized mice. Because eosinophil immigration into the airways and infiltration into the interstitium of the lungs is a prominent feature of human and experimental ABPA, many studies have addressed the requirements for eosinophil accumulation as an integral part of the disease. Wild type mice exposed intranasally to *A. fumigatus* antigens develop not only lung eosinophilia but also blood and bone marrow eosinophilia [188, 190, 208–210]. If these animals are treated with neutralizing antibodies to IL-5, the bone marrow, blood, and lung eosinophilia is abolished [194, 201, 209]. The essential requirement for IL-5 has also been demonstrated with many other immunogens, for example, parasites and ovalbumin [211]. GM-CSF is another cytokine that can activate and maintain eosinophils in the tissues. GM-CSF-producing T cells have been shown to accumulate in the lungs of animals exposed to *A. fumigatus* antigen [195]. However, GM-CSF must be less important than IL-5, because endogenous GM-CSF production cannot support eosinophilia in animals treated with neutralizing anti-IL-5 antibodies [189, 194, 209, 211] or in IL-5 gene knockout mice [212].

The role of chemokines and adhesion molecules in mediating lung eosinophilia has not been investigated thoroughly in experimental ABPA. It has been reported that mRNA for RANTES is up-regulated after sensitization of mice with *A. fumigatus* antigen [189]. It will be of great interest to determine the role of eotaxin, because it has been shown to be a major chemoattractant for eosinophils in the lungs [213]. Macrophage inflammatory protein-1 α , RANTES, and MCP-4 are other chemokines that may be instrumental in eosinophil recruitment to the lungs [188, 214–216]. The ability of eotaxin to induce eosinophil accumulation is still dependent on IL-5, because the infusion of eotaxin at a dose that will elicit eosinophilia in wild type mice does not cause eosinophilia in IL-5-deficient animals [217].

Eosinophils have been shown in vitro to secrete mediators that are proinflammatory or cytotoxic. In experimental ABPA,

eosinophils do not appear to be critical in mediation of tissue injury, because IL-5-deficient animals or animals treated with anti-IL-5 antibodies still develop the full spectrum of microscopic lesions [194, 203]. The infiltrates, consisting of mononuclear cells instead of eosinophils, appear to be just as substantial as in wild type mice. The changes in the airway epithelium (goblet cell hyperplasia) are still present to the full extent. Furthermore, eosinophils are not required to mediate airway hyperreactivity, because IL-5-deficient animals or mice treated with neutralizing anti-IL-5 antibodies develop airway hyperreactivity similar to that in wild type mice when exposed to *A. fumigatus* antigens. Thus, in experimental ABPA, the consequences of lung eosinophilia are still unclear, although eosinophils may be at least partly responsible for tissue injury.

T cell responses in mice. The role of T cells in experimental ABPA has also been investigated [203]. Mice with targeted disruption of the recombinase-activating genes (RAGs) were studied to examine the roles of T or B cells. Because recombinase is critically involved in the gene rearrangement of immunoglobulins and TCRs and these molecules in turn are critically important for B and T cell development, RAG-deficient mice are devoid of mature B and T cells. After exposure to *A. fumigatus* antigens, RAG-deficient mice failed to develop significant lung lesions or airway hyperreactivity. When RAG-deficient mice are reconstituted with highly purified CD4⁺ T cells, sensitization will induce severe eosinophilic inflammation of the lungs. These mice also developed airway hyperreactivity [203]. Thus, CD4⁺ T cells are essential for the development of peribronchial, perivascular, and alveolar lesions and also for airway hyperreactivity in experimental ABPA [203]. The data obtained with *Aspergillus*-exposed RAG-deficient mice are still surprising, because the components of the *A. fumigatus* antigen extract are thought to be able to induce tissue injury and activate cells of the innate immune system. A substantial number of eosinophils can be found in BAL fluid from RAG-deficient mice exposed to *A. fumigatus* antigens (~20- to 30-fold more than in unchallenged mice, and ~3- to 5-fold less than in sensitized wild type mice) [203].

As predicted from the predominant IgE and IgG1 responses induced by sensitization with *A. fumigatus*, the dominant T cell response is of the Th2 type [90]. The T cell response has been analyzed by different methods. Cell suspensions from the lungs, draining lymph nodes, or spleens have been restimulated in vitro with immobilized anti-CD3 antibodies or with crude antigen extract. Cell suspensions from sensitized but not from control mice produce significant amounts of Th2 cytokines, such as IL-4, IL-5, IL-6, and IL-10 [90, 218]. The Th1 cytokine IFN- γ , on the other hand, is made in equivalent amounts by cells from controls and sensitized mice. Similar results have been obtained from the analysis of lung T cells for the presence of intracellular cytokines or from the examination of BAL fluid

for secreted cytokines [218]. Compared with controls, sensitized animals have a larger number of Th2 lymphocytes in the lung tissue, and antigen challenge induces the release of Th2 cytokines into the airway lumen. Immunohistochemical analysis has shown that exposure to *A. fumigatus* antigens induces peribronchial and perivascular infiltration of T cells that make IL-4 or IL-5 [195].

Little is known about T cell migration and homing in the lungs in experimental ABPA. Intercellular adhesion molecule 1 (ICAM-1), an adhesion molecule of the immunoglobulin superfamily, may be important, because the number of T cells in lung tissues of mice exposed to *A. fumigatus* antigens was positively correlated with ICAM-1 expression [196].

Lung inflammation. The response in mice depends on the route of exposure and prior sensitization. Exposure of mice to *A. fumigatus* antigens induces a strong eosinophilic inflammation in the lungs that persists over several days [188–191, 193–195, 202, 203, 209, 210, 219]. Microscopic changes differ in severity depending on the route of sensitization, the frequency of sensitization, the form of the antigen, and the mouse strain. Pathological lesions tend to be multifocal in less severely affected mice but coalesce and become more diffuse in severely affected animals. Airway lesions are characterized by emigration of eosinophils and mononuclear cells into the lumen, goblet cell hyperplasia, and, in severe cases, epithelial metaplasia and mucus accumulation. Inflammatory infiltrates are found in the interstitial tissues of the airways, blood vessels, and alveoli. They consist primarily of lymphocytes and eosinophils, accompanied by smaller numbers of macrophages, plasma cells, and neutrophils. In cases of severe lung inflammation, alveolar septa in affected areas are thickened by inflammatory cells. Accumulation of collagen is frequently noted at the subepithelial area. Alveolar inflammation in these areas becomes granulomatous with the accumulation of multinucleated giant cells. When mice are examined 3 or 4 weeks after the last antigen exposure, the vast majority of the microscopic lesions have disappeared. In most instances, the lung lesions are reversible; however, chronic exposure to antigen may lead to fibrosis and collagen deposition and irreversible lung damage.

Analysis of BAL fluid reveals that naive mice have a strong neutrophil and macrophage response after the first intranasal exposure to *A. fumigatus* antigens [193]. After few additional exposures, >50% of the cells in the BAL fluid are eosinophils, and the remainder are small mononuclear cells, macrophages, and, rarely, neutrophils. The infiltrates within the lung tissue are dominated by eosinophils at that time. If intranasal exposures to *A. fumigatus* antigens are continued for a prolonged period of time, numbers of mononuclear cells in the BAL fluid increase and numbers of eosinophils decrease, although they remain well above those in saline-treated controls. In the lung tissues, the accumulation of mononuclear cells is more prom-

inent than of eosinophils. In some mouse strains, multiple exposures to *A. fumigatus* antigens over a prolonged time period will lead to an attenuation of the histological lesions, in contrast to the acute response induced after a few exposures [184].

Airway hyperreactivity. Sensitization with *A. fumigatus* antigens consistently induces very strong airway hyperreactivity [202, 203, 218]. The relative potency of *A. fumigatus* antigens in inducing airway hyperreactivity appears to be increased compared with that of other antigens, such as ovalbumin. For example, *A. fumigatus* antigens have been successfully used to induce airway hyperreactivity in all of the inbred strains of mice tested thus far (C57BL/6, 129SvEv, BALB/c, and crosses of these strains). The presence of *Aspergillus*-specific antibodies or marked eosinophilia has no role in determining the airway response in the antigen-exposed mice. IgE, IL-4, and IL-5 knockout mice showed airway responses comparable to those of the wild type mice exposed similarly to *A. fumigatus* antigens [202, 203]. All of the RAG $-/-$ animals sensitized with *A. fumigatus* failed to show any airway hyperreactivity. However, RAG $-/-$ mice reconstituted with CD4⁺ cells regained airway response, indicating the major role played by T cells in inducing airway response [203].

Airway remodeling. Little attention has been paid to the airway remodeling, airway barrier, and tight junction proteins in the development of the disease and the reversibility of the responses. Mucosal defense of the airways of healthy persons is a highly efficient barrier in eliminating insulting antigens and microbes. The physical barrier prevents the penetration of large molecules and particles. The particles are frequently removed by phagocytic cells. The epithelial layer secretes mucus, particularly by the goblet cells, and eliminates the impacted particles and microbes. The tight junctions retain the integrity of the airway epithelium and actively prevent the transport of different molecules between the epithelial cells and across the epithelium. The secretory cells present in the epithelial cell layer and their products, such as mucin, defensive proteins, and enzymes, together constitute a protective environment in the airway [91]. An important function of the epithelial lining fluid of the airways is the elimination of inhaled particles, for example, fungal spores, that have entered the lung and were impacted on the mucosal surface. By the coordinated ciliary movement of specialized epithelial cells, the epithelial lining fluid, together with impacted material, is transported out of the airways into the oropharyngeal cavity. The clearance is dependent on such factors as drugs that activate the adenylyl cyclase system and mediators that are released during infections and asthmatic inflammatory responses [220]. The proteins excreted by epithelial cells and/or mucosal gland cells have the ability to kill potential pathogens, for example, bacteria, viruses, and fungi. These proteins mainly belong to the family of the cationic molecules, such as defensins and secretory leukoprotease, that kill both

bacteria and fungi and inhibit serine proteases, contributing to the innate defense of the host [221, 222]. Another level of defense of the airways is effected by functional families of proteins, such as C-type lectins, glycolipids, and glycoproteins, that are able to opsonize microorganisms by binding to surface structures [223]. One of the important functions of these glycoproteins is to prevent the attachment of microorganisms to the epithelial surface, which is the first and most important step for a pathogenic microorganism to gain access to its host.

Aspergillus antigen introduced via the intranasal route caused airway destruction in 24–48 h with a large number of periodic acid–Schiff–positive goblet cells. The integrity of the epithelium is disturbed, and the basal membrane becomes more thickened and shows the presence of collagen deposits [188, 203, 218]. The intercellular tight junctions became discontinuous in 24 h after the antigen challenge and were further disrupted. Histochemical staining for occludin and ZO1 proteins indicated marked destruction in antigen-exposed mice. Thus, it can be concluded that the airway remodeling is the result of antigenic components and other biochemical components, including enzymes, present in the antigen. By use of some of the recombinant proteins without enzyme activity, and by inactivating the enzyme activity of *A. fumigatus* antigen, airway remodeling was arrested in mice (V. B. Rathore and V.P.K., unpublished data). The role of chemokines in airway remodeling has been demonstrated in a mouse model [215].

Cytokines in murine models of ABPA. Mice exposed to *A. fumigatus* antigen developed elevated levels of IgE and eosinophilia in the peripheral blood, bone marrow, and lungs [90, 192, 207]. High IgE production in response to *Aspergillus* challenge has been attributed to the expression of IL-4 by activated lymphocytes belonging to CD4⁺ Th2 cells. Consistently exaggerated production of IgE and IgG1 has been reported in mice exposed to *Aspergillus* antigens [90, 190]. The IgE response was arrested or retarded by the administration of anti-IL-4 antibody. However, no significant difference was detected in the levels of IgG1. A marked increase in IgG2a antibody levels in these animals was also observed, indicating that anti-IL-4 antibody induced a more pronounced Th1 response, because IgG2a levels are IFN- γ dependent [194]. Thus, anti-IL-4 antibody treatment resulted in a suppressed Th2 response and an enhanced Th1 response. Similarly, a Th1-type response was also detected in IL-4 knockout mice challenged with *Aspergillus*. In these mice, no IgE was demonstrable after *Aspergillus* challenge, but considerable enhancement in the levels of IgG2a was detected [189, 194].

It has been shown that neutralizing anti-IL-5 monoclonal antibody injected ip partially abrogated the eosinophilia in all 3 compartments, namely peripheral blood, lung, and bone marrow, suggesting a major role for IL-5 in eliciting eosinophilia [194]. Multiple anti-IL-5 antibodies were effective in main-

taining baseline levels of blood eosinophils. Injection of multiple anti-IL-4 antibodies also down-regulated eosinophils in bone marrow, lungs, and peripheral blood, although to a lesser extent than in mice injected with anti-IL-5 antibody [194]. This reduction in eosinophil numbers by anti-IL-4 antibody treatment may be caused by the down-regulation of IL-5 production because of the reduction of Th2 cells in these mice.

Observations with IL-4 knockout mice suggest that the lung injury observed in this instance may be due to eosinophil mediators and IFN- γ but not to IL-4 and IgE [189]. In this model, eosinophil infiltration in the lung tissue was observed, whereas, surprisingly, no mRNA for IL-5 was detected. These mice expressed mRNA for RANTES, suggesting a role for this chemokine in the recruitment of eosinophils. It is possible that eotaxin and GM-CSF also may be involved in the recruitment of eosinophils, because IL-4-induced eotaxin production by fibroblasts has been reported [224].

Very little is known about mediators that down-regulate the immune response to *A. fumigatus* antigens. IL-10 is known to be a potent immunosuppressive molecule. It is constitutively produced by bronchial epithelial cells. IL-10 has been shown to inhibit Th1 responses in mice and cytokine production of Th1 and Th2 lymphocytes in humans [225]; it is thought to exert its suppressive function by inhibiting antigen-presenting cells, such as dendritic cells or macrophages. In experimental ABPA, the major role of endogenous IL-10 is to inhibit lung inflammation [218]. In a mouse (cross of C57BL/6 and 129SvEv) that developed a mixed T cell response (Th2 and some Th1), endogenous IL-10 prevented mortality and restricted the secretion of IL-5 and IFN- γ into the airway lumen. In this model, production of IFN- γ was detrimental to the IL-10-deficient animals, because neutralizing antibodies to IFN- γ reduced mortality. In C57BL/6 mice primed ip and challenged intranasally with *A. fumigatus* antigen, endogenous IL-10 limited IL-5 production and lung eosinophilia. However, in the same mouse strain (C57BL/6), endogenous IL-10 had no detectable effect on the response to intranasal exposure to *A. fumigatus* antigens, most probably because of compensatory mechanisms. IL-10 may be beneficial in restricting the inflammation induced by inhalation of *A. fumigatus* antigens [218].

As shown above, both IL-4 and IL-13 contribute to the allergic phenotype. After exposure of mice to *A. fumigatus*, both IL-4 and IL-13 show significant increases in 30 days. IL-13R α 1 was shown to be elevated in these mice, but IL-13R α 2 constitutively expressed in naive lung was absent in *A. fumigatus*-sensitized mice. Neutralization of IL-13 resulted in reduced airway inflammation and hyperreactivity, whereas the response was not as remarkable after immunoneutralization of IL-4 [226]. Furthermore, goblet cell hyperplasia was inhibited by anti-IL-13 treatment, whereas anti-IL-4 treatment failed to show any effect. An anti-IL-4-induced response was induced

through the expression of Th1 cytokines IFN- γ and IL-12, whereas IL-13 neutralization did not result in enhanced IFN- γ or IL-12 production and, hence, may not follow the systemic suppression pathway exhibited by anti-IL-4 treatment. Unpublished results (V.P.K., D. B. Corry, G. Grunig, A. Hadeiba, M. L. Warnock, D. Sheppard, D. M. Rennick, R. M. Locksley) indicate that RAG $-/-$ mice reconstituted with T cells followed by sensitization with *A. fumigatus* and neutralization of IL-13 failed to show any suppression of lung inflammation. However, sensitized wild type mice neutralized with anti-IL-13 reduced the lung inflammation (D. Rennick, G. Grunig, J. C. Ford, D. D. Donaldson, R. Verkayya, C. McArthur, E. Hansell, V.P.K., M. Warnock, unpublished data). In results obtained with reconstituted T cells in double-knockout mice (IFN- γ $-/-$ and RAG $-/-$), it was found that anti-IL-13 suppressed the pulmonary and airway responses through an IFN- γ -dependent pathway.

Role of chemokines in ABPA. As has been shown above, a number of cytokines participate in the pathogenesis of ABPA [188, 194, 214, 215]. The role of chemotactic cytokines in allergic aspergillosis is not fully understood. Asthmatic patients challenged with allergens show elevation of C-C chemokines, such as MCP-1, RANTES, eotaxin, and macrophage inflammatory protein-1 α [194, 195, 214, 215, 227]. Extended studies indicate that, despite the fact that chemokines are produced by a number of cells, functional redundancy among chemokines in experimental allergic airway responses is minimal. Recently, a number of studies evaluated the role played by chemokines and chemokine receptors in the pathogenesis of allergic aspergillosis [188, 214, 215, 227]. BAL fluid from mice challenged with *A. fumigatus* demonstrated chemokines C10 and MCP-1 by ELISA, but the increased presence of C10 was remarkable. C10 was produced by vascular smooth muscle cells and by fibroblasts, although alveolar macrophages produced C10 in much larger quantities when treated with IL-4 but not with other cytokines [227]. C10 was found to be a moderate eosinophil chemoattractant in the lungs. However, animals treated with anti-C10 antibody markedly reduced the eosinophils in BAL fluid, whereas no difference was noticed with neutrophil and macrophage numbers. A significant reduction in lymphocytes was also detected in the BAL fluid of mice challenged with *A. fumigatus* and injected with anti-C10 antibody. The immunoneutralization of C10 also attenuated the MCP-1 and eotaxin levels in the BAL fluid and in the whole lung homogenate. High levels of IL-13 produced in *A. fumigatus*-sensitized mice were also attenuated by the administration of anti-C10 antibody. The airway hyperreactivity noted in the *A. fumigatus*-sensitized mice can be arrested by treating the animals with anti-C10 antibodies. Thus, in the overall pathogenesis of ABPA, C10 plays a major role.

The role of chemokine receptors CCR1 and CCR2 in the

pathogenesis of ABPA has been investigated [214, 215]. *A. fumigatus*-sensitized CCR1 wild type (+/+) and CCR1 knockout (-/-) mice demonstrated similar serum IgE and polymorphonuclear leukocyte numbers in BAL fluid. In the CCR1 -/- mice, IFN- γ levels in the lung were significantly higher than in wild type mice, and only an attenuated Th2 response was detected compared with that in wild type mice challenged with *A. fumigatus* [215]. IL-4, IL-13, Th2-inducible C10, eotaxin, and macrophage-derived chemokines were significantly lower in CCR1 -/- than in CCR1 +/+ mice. These results suggest that CCR1 is a major contributor to the pathogenesis of ABPA. Because the inflammatory responses have been shown to be much less in CCR1 -/- mice, with fewer goblet cells and less subepithelial fibrosis, CCR1 may be a contributor to the airway remodeling from the inflammatory response caused by *A. fumigatus* allergen.

The role of CCR2, the receptor for MCP-1, was investigated with use of knockout mice [214]. Knockout mice lacking CCR2, when exposed to antigens of *A. fumigatus*, showed major defects in the recruitment of polymorphonuclear leukocytes but invariably showed significant elevations in eosinophils and lymphocytes in BAL fluid. These mice had significant increases in serum IgE and whole lung levels of IL-5, IL-13, eotaxin, and RANTES compared with levels in CCR2 +/+ mice. Similarly, airway inflammation, hyperresponsiveness to spasmogens, and subepithelial fibrosis were significantly enhanced in CCR2 -/- mice compared with CCR2 +/+ mice after *A. fumigatus* antigen challenge. Thus, CCR2 plays an important role in the immune response against *A. fumigatus* by limiting the pulmonary airway inflammation and remodeling responses to *A. fumigatus*.

Recombinant *A. fumigatus* allergens in murine model.

The majority of the animal model studies of ABPA have been carried out in mice with use of crude *A. fumigatus* or with intact organism, particularly the spores [4, 184]. The results of these studies yielded valuable information on the role played by various cytokines, eosinophils, IgE, chemokines, and T cell differentiation and signaling in disease development [4, 184, 188, 194, 227]. In recent years, a number of relevant allergens from *A. fumigatus* have been identified, the corresponding cDNA has been cloned and sequenced, and the encoding allergens have been expressed [8, 78, 135, 136, 228–235] (see Recombinant Allergens for the Diagnosis of ABPA). Several of these allergens bound specifically to serum IgE from patients with ABPA, and their disease specificity has been established [8, 135, 231]. However, none of these major allergens have been investigated in the murine model to determine their specific roles in the pathogenesis of ABPA. Studies with the purified allergens may contribute to our understanding of the specific roles of the individual allergens. By understanding the structure-function relationship of the allergens, and with the knowledge of the immunopathogenesis of ABPA, it is possible to

develop a vaccine or immunotherapeutic agent to manage the disease [236].

Four major allergens of *A. fumigatus* (Asp f1, f3, f4, and f6) were investigated in a murine model [236]. Mice exposed to Asp f1, f3, and f4 showed inflammatory changes in the lungs and airway hyperreactivity. The immune responses demonstrated include elevated serum IgE; marked production of allergen-specific IgE; peripheral blood, lung, and BAL fluid eosinophilia; and cytokines typical of a Th2 type. Asp f6 failed to show any airway hyperreactivity and invariably showed less inflammatory response in the lungs and the overall truncated antibody response. In another study, Asp f2 was shown to follow the same pattern of Asp f6; however, when combined with Asp f13, an alkaline serine proteinase, it provoked marked inflammatory, immune, and airway responses. Thus, it is clear that such studies may yield more useful information on the individual allergen and the cumulative effect of different components when challenged with allergens. These studies will lead to a better definition of the mechanism of the disease, which may eventually contribute to the initiatives for controlling the disease.

Immunotherapy. Specific immunotherapy and vaccination are the best probable means for controlling type I allergy. Earlier studies concentrated on crude allergens as immunotherapeutic agents. In recent years, with our clearer understanding of the pathogenesis of the disease, attempts have been directed to reverse the Th2 type of response to a Th0 or a Th1 response. There are currently several major directions in immunotherapy of IgE-mediated allergy, including peptide immunotherapy, naked DNA vaccination, and immunotherapy with use of ISS-ODN.

Allergen-specific therapy may aim for prophylaxis of atopy, the induction of tolerance, or to the modification of ongoing immune responses [237–239]. Although attempts have been made with other allergens to induce T cell nonresponsiveness in patients by selectively administering major T cell epitopes, no such studies have been carried out with *A. fumigatus* allergens. Asp f2, a major *A. fumigatus* allergen, was studied and a number of deletion and point mutants obtained [240]. Some of these mutated proteins, although eliciting T cell responses in patients, failed to bind to IgE in serum from mice immunized with the allergen. Thus, candidate allergens for immunotherapy need further evaluation.

During the study of synthetic peptides representing sequences of Asp f1, another major allergen from *A. fumigatus*, 2 peptides were identified (peptide 5 and peptide 10) with cytokine specificities. Peptide 5 (NGYDGNGLIKGRTP) elicited a Th2-type cytokine response and peptide 10 (KVFCGI-VAHQQRGN) a Th1-type cytokine response when used in immunizing mice [241]. Mice immunized with 2 other epitopes of Asp f1 by the iv route were prevented from elaborating an

immune response when they were subsequently challenged with *A. fumigatus* crude antigen [239]. These results support a potential role for synthetic peptides as immunotherapeutic agents in allergic aspergillosis, but they need further study.

Immunostimulatory sequences and vaccination. A number of studies have appeared in recent years emphasizing the usefulness of immunostimulatory cytosine polyguanine (CpG) oligonucleotides (ISS-ODN.CpG-ODN) in vaccination against IgE-mediated allergy [242]. Immunization with DNA-based vaccines, such as plasmid DNA of major *A. fumigatus* antigens, or the allergen and ISS-ODN coadministered resulted in reversal to a predominantly Th1-based immune response instead of the usual Th2 type of immune response. BALB/c mice were immunized ip with alum-precipitated *A. fumigatus* culture filtrate antigens at 3-day intervals 3 times. This was followed by 2 intranasal exposures at 5-day intervals, and animals were sacrificed 24 h after the last antigen challenge. For immune intervention, a second group of mice was immunized first with ISS-ODN sequence (50 µg/animal) and then with ip *A. fumigatus* antigen. Two more doses of ISS-ODN were administered between the intranasal challenges. A third group of animals received only 1 dose of ISS-ODN (35 µg/animal) after the last ip antigen immunization. On sacrifice, sera were studied for specific antibodies and peripheral blood for eosinophils. The lung histology, mucoglycoconjugates in the airway epithelium, and antigen-induced cytokine expression in lung compartments were investigated. There was a 3-fold increase in serum IgG2a in the ISS-treated group compared with levels in mice treated with antigen alone. Blood eosinophil counts of the ISS-treated group were significantly lower than those of the antigen-immunized group. Pulmonary histology revealed significantly less eosinophilic infiltration in the perivascular and peribronchial regions of CpG-ODN-treated mice. Periodic acid-Schiff staining of lung sections also indicated enhanced mucoglycoconjugate production in mice without CpG compared with that in CpG-injected animals. These results suggest that the immune deviation induced by CpG motifs may alter the Th2-type allergic responses in *A. fumigatus*-sensitized mice and, hence, that CpG may be a possible candidate for immunotherapy as one element of future treatment of allergic aspergillosis [242] (B. Banerjee and V.P.K., unpublished results).

Conclusion and future directions. The animal model studies of ABPA are summarized in tables 3 and 4. The results of the murine model studies clearly demonstrate that CD4⁺ Th2 cells are critical for induction of *A. fumigatus*-induced allergy. However, no animal model comparable to ABPA in CF is currently available. Although the major features of ABPA in CF and asthmatic ABPA show similarities, they show differences as well. Hence, any translation of the animal model results to CF and ABPA should be approached carefully, after evaluation of the studies. In experimental ABPA, along with the Th2 re-

sponse, a mild to moderate Th1 immunopathologic response can occur. Whether this is due to the antigen specificity or to genetic or other factors is not well understood. The antibody response, eosinophil differentiation and chemotaxis, and T and B cell infiltration and activation are part of the overall response detected in the model and are comparable to factors in human ABPA. The cytokines, chemokines, their receptors, and various other factors produced by cells of the immune system are also needed for the full expression of the disease. Thus, a clear understanding of the mechanism may help in effectively controlling progression by altering or stopping the regulatory signals. However, the various factors, including cytokines and chemokines, could be produced by different cell types, and hence a multifaceted attempt may prove successful in controlling the disease. Airway remodeling, inflammation, and hyperresponsiveness should be considered when immunotherapeutic approaches are considered.

CLASSIC ABPA AND CRITERIA FOR DIAGNOSIS

In patients with persistent asthma, the use of immediate skin testing with *Aspergillus* species followed by serological testing helps identify patients with ABPA with central bronchiectasis or patients with ABPA and seropositivity (ABPA-S) who do not have current symptoms or signs [39]. Immediate cutaneous reactivity to *Aspergillus* species is detectable in 20%–25% of patients with persistent asthma [13]. Cutaneous reactivity testing is an inexpensive, rapid, easy, and highly sensitive screening test with high negative predictive value, which is followed by a serological battery of assays with high specificity [243]. The latter include assays of total serum IgE concentration (assay cutoff value for a positive result, >417 IU/mL or >1000 ng/mL), serum precipitins to *A. fumigatus*, and serum IgE and IgG antibodies to *A. fumigatus*.

Diagnostic criteria. The classic case of ABPA fulfills the following criteria [244–246]: (1) asthma; (2) chest roentgenographic infiltrates—current or in the past—may be detectable on CT when chest radiography is unremarkable; (3) immediate cutaneous reactivity to *Aspergillus* species; (4) elevated total serum IgE (>417 IU/mL or >1000 ng/mL); (5) serum precipitating antibodies to *A. fumigatus*; (6) central bronchiectasis on chest CT; (7) peripheral blood eosinophilia; and (8) elevated serum IgE and/or IgG to *A. fumigatus*.

It has been suggested that the minimal essential criteria for diagnosis of ABPA include the following [246]: (1) asthma; (2) immediate cutaneous reactivity to *Aspergillus* species; (3) elevated total serum IgE concentration (>417 IU/mL or >1000 ng/mL); (4) elevated serum IgE to *A. fumigatus* and IgG to *A. fumigatus*; and (5) central bronchiectasis. The presence of precipitating antibodies to *A. fumigatus* was found to support the

Table 3. Summary of animal models of allergic bronchopulmonary aspergillosis.

| Mouse type, treatment | Serum IgE | Lung inflammation | Airway hyperreactivity | Cytokine response |
|--------------------------------------|-----------|----------------------|------------------------|---------------------------|
| Wild type | | | | |
| None | +++ | +++ | +++ | IL-4, IL-5 |
| Anti-IL-5 | +++ | ++ | +++ | NT |
| Anti-IL-4 | – | ++ | – | IFN- γ |
| Knockout mice, anti-IFN- γ | | | | |
| IL-4 $-/-$, none | | | +++ | IFN- γ |
| IL-5 $-/-$, none | ++ | +++ | – | IFN- γ |
| IL-10 $-/-$, none | +++ | ++++ | +++ | IL-4, IL-5, IFN- γ |
| IL-13 $-/-$ | | | | |
| None | ++ | +++ | +++ | IL-4, IL-5, IFN- γ |
| Anti-IL-5 | ++ | ++ | ++ | IFN- γ |
| Anti-IL-4 | – | ++ | + | IFN- γ |
| IgE $-/-$, none | | | | |
| IgG1 $-/-$, none | ++ | +++ | +++ | IL-4, IL-5, IFN- γ |
| B cell $-/-$ | | | | |
| None | – | +++ | +++ | IL-4, IL-5 |
| Anti-IL-5 | – | +++ (no eosinophils) | +++ | NT |
| Anti-IL-4 | – | ++ | – | NT |
| RAG $-/-$ | | | | |
| None | – | + | – | – |
| Transfer of CD4 ⁺ T cells | – | ++++ | + to ++ | IL-4, IL-5, IFN- γ |
| C10 $+/+$ (wild type mice) | | | | |
| None | ++ | +++ | +++ | IL-4, IL-5, IL-13 |
| Anti-C10 | ++ | ++ | – | – |
| CCR1 $+/+$ (wild type mice), none | | | | |
| CCR1 $-/-$, none | ++ | + | – | IFN- γ |
| CCR2 $+/+$ (wild type mice), none | | | | |
| CCR2 $-/-$, none | ++ | +++ | +++ | IL-4, IL-5, IL-13 |

NOTE. CCR, CC chemokine receptor; NT, not tested; RAG, recombinase-activating gene; $+/+$, wild type; $-/-$, knockout; –, none; +, slight; ++, mild; +++, moderate; +++++, heavy.

diagnosis of ABPA, and in patients with ABPA, total IgE levels were found to predict disease exacerbations. Even these criteria may be too rigid, because use of prednisone or other oral corticosteroid agents can reduce the total serum IgE concentration to <1000 ng/mL in some patients with ABPA.

Other diagnostic elements that may be viewed as supportive of the diagnosis include a history of coughing up either mucus plugs or sputum flecked with brown, black, or green elements; culture of sputum yielding *A. fumigatus* or a sputum smear in which *A. fumigatus* elements were identified microscopically; a sputum smear showing eosinophils; or a chest radiographic sign suggesting bronchial inflammation with or without plugs (“ring sign,” indicating only bronchial wall thickening; “parallel lines” or “tram tracks,” suggesting bronchiectasis, because the normal bronchus tapers and therefore does not have parallel walls; or “glove-finger,” suggesting mucus impaction).

Staging of ABPA. Although not applied often in the case

of patients with ABPA and CF, a staging system has been used for patients with asthma and ABPA [247]. There are 5 stages in patients with central bronchiectasis and 4 stages in patients without bronchiectasis [39]. The stages are not phases of disease; patients need not pass from one stage to the next in some orderly or predetermined progression. Stage I is the acute stage, in which there are chest roentgenographic infiltrates, usually of upper lobes or the middle lobe, associated with a markedly elevated total serum IgE concentration and peripheral blood eosinophilia. The infiltrates clear with prednisone treatment, and prednisone can be discontinued [247]. Treatment may be required for intermittent or persistent asthma, however. Stage II is “remission,” referring to patients who have not had chest roentgenographic infiltrates or need for prednisone for at least 6 months. Some patients may enter seemingly a permanent remission or, in fact, “cure” of ABPA. Stage III is recurrent exacerbation and is similar in clinical and chest roentgeno-

Table 4. Immune components in animal models of allergic bronchopulmonary aspergillosis.

| Features | Features involved in allergic response | Features suppress allergic response |
|----------------------|--|-------------------------------------|
| Antibody | | |
| IgE | Yes | No |
| IgG1 | Yes | No |
| IgG2a | No | Yes |
| Lymphocytes | | |
| CD4 ⁺ Th1 | No | Yes |
| CD4 ⁺ Th2 | Yes | No |
| Cytokines | | |
| IL-4 | Yes | No |
| IL-5 | Yes | No |
| IL-13 | Yes | No |
| IFN- γ | No | Yes |
| IL-12 | No | Yes |
| IL-18 | No | Yes |
| Chemokines | | |
| RANTES | Yes | No |
| Eotaxin | Yes | No |
| C10 | Yes | No |
| Chemokine receptors | | |
| CCR1 | Yes | No |
| CCR2 | No | Yes |

NOTE. CCR, CC chemokine receptor; RANTES, regulated on activation, normally T cell-expressed and stimulated cytokine.

graphic presentation to stage I, with elevated total serum IgE levels, typically at least 100% greater than non-stage III baseline concentrations. The chest roentgenographic infiltrates clear with prednisone therapy, or the results of plain film or high-resolution CT return to a pattern of limited scarring. Stage IV is steroid-dependent asthma, in which there may or may not be subsequent roentgenographic infiltrates. The total serum IgE concentration varies greatly in such patients, for example, 165–15,000 ng/mL, depending on whether there is a pulmonary infiltrate [247]. Prednisone and inhaled corticosteroids are necessary for management. Stage V is fibrotic disease, diagnosed on the basis of chest roentgenographic or high-resolution CT findings, irreversible impairment in pulmonary function, and inadequate response to prednisone [247]. Total serum IgE concentrations in 1 report ranged from 164 to 41,213 ng/mL and in another from 360 to 25,000 ng/mL [248]. Some patients survive with marked respiratory impairment and harbor *Pseudomonas* species in sputum and/or sinuses. Fatalities have occurred in this group [248]. The total serum IgE concentration was >1000 ng/mL in 15 of 17 patients in stage V [248]. Cavities were present on chest radiographs of 5 of 17 patients, with chronic interstitial infiltrates in all. Five patients died of res-

piratory failure; for all of them, the fixed expiratory volume in 1 s (FEV₁) 6 months after cessation of prednisone treatment was <0.8 L [248]. Patients with end-stage lung disease in stage V share features with patients with CF, although sweat tests (assays of sweat electrolytes) yield negative results, there is a lack of infertility, the subjects are older, and there is a lack of pancreatitis in nearly all cases. However, there may be bronchial colonization with *Pseudomonas* species, extensive bronchiectasis and pulmonary fibrosis, need for supplemental oxygen, and progressive decline in respiratory status.

Patients with ABPA-S may be classified into stages I–IV but do not have irreversible fibrosis or end-stage respiratory disease [39]. These patients may have recurrent infiltrates and be categorized as stage III. On the basis of clinical, spirometric, serological, and radiological findings, these patients have a milder type of ABPA. Indeed, in a study of itraconazole treatment for ABPA, one conclusion was that improvement was more likely to occur in patients without bronchiectasis than in those with it (43% vs. 20%) [249].

Comment. Many publications do not use the above staging system, making analysis of literature more difficult. Often, patients with ABPA-S are not identified in series. These patients appear to be an important population for genetic analysis (literature consensus grade III; table 5).

DIAGNOSIS OF ABPA IN CF

Predisposing Factors for ABPA in CF

Other pulmonary infections. An increase in the frequency and severity of bacterial lung infections in patients with CF will usually lead to an increased use of antibiotics, which has been suggested to “pave the way” for fungal infection [251]. The use of inhaled tobramycin may lead to an increased rate of isolation of *Aspergillus* species [251]. However, another prospective study of inhaled colistin failed to show increase in fungal colonization of sputum [252].

Genetics. Patients with ABPA but without CF have been demonstrated to have higher frequencies of *CFTR* mutations than are found among subjects with bronchitis or in the normal population [183]. It has been suggested that HLA-DR molecules DR2, DR5, and possibly DR4 or DR7 contribute to susceptibility, whereas HLA-DQ2 contributes to resistance, and a combination of these may determine the outcome of ABPA in CF and asthma [105, 253].

Immune Responses in CF Relevant to ABPA

It has been suggested that atopy is an important risk factor for the development of ABPA in patients with CF [12, 35]. Atopy (defined as >1 IU/mL IgE antibody to >1 allergen) has been shown to be present in 61% of 104 tested patients with CF, and ABPA was diagnosed in 9% [35]. ABPA occurred in 22%

Table 5. Literature consensus grades of evidence.

| Grade | Explanation |
|-------|---|
| I | Evidence obtained from at least 1 properly randomized, controlled trial |
| II-1 | Evidence obtained from well-designed controlled trials without randomization |
| II-2 | Evidence obtained from well-designed cohort or case-control analytic studies, preferably from >1 center or research group |
| II-3 | Evidence obtained from multiple time series with or without the intervention; dramatic results in uncontrolled experiments (such as the results of the introduction of penicillin treatment in the 1940s) could also be regarded as this type of evidence |
| III | Opinions of respected authorities, based on clinical experience, descriptive studies, or reports of expert committee |

NOTE. From [250].

of atopic patients with CF but in only 2% of nonatopic patients. Fifteen of 16 patients with ABPA were atopic, as defined by skin test positivity to at least 1 common aeroallergen other than *A. fumigatus*. Patients with ABPA demonstrate patterns of response to *A. fumigatus* different from those to other allergens [17, 35]. For example, in a group of 75 patients with CF, immediate cutaneous reactivity to *A. fumigatus* or other allergens occurred in 43% versus isolated reactivity to *A. fumigatus* in 15% [17]. Overall, immediate cutaneous reactivity to *A. fumigatus* was present in 44 patients (59%). The total serum IgE concentration was >540 ng/mL in 12 (16%). Increased serum IgE and/or IgG to *A. fumigatus* was detected in 38 subjects (51%). In contrast to the case in commercially performed determinations, the control sera were from patients with asthma and immediate cutaneous reactivity to *A. fumigatus* but in whom sufficient criteria for ABPA (or CF) were not present. Precipitating antibodies to *A. fumigatus* were recognized in 42% of subjects, and peripheral eosinophilia (>500/ μ L) was observed in 29% [16].

Hamilton and Carswell [254] found that type I sensitivity to *A. fumigatus* developed earlier than sensitivity to other allergens in patients with CF and that positive skin test reactions to molds were associated with delayed onset of *P. aeruginosa* colonization. A regression analysis of epidemiological data from >14,000 patients with CF indicated that the presence of wheezing, bronchial asthma, or *P. aeruginosa* in sputum increases the relative risk of ABPA by >1.5 times [26]. The presence of *P. aeruginosa* in cultures of sputum was, however, of only borderline significance when other risk factors were present in the model [26]. In a much smaller but prospectively conducted study of 156 patients with CF, the frequency of *A. fumigatus* was independent of presence as well as duration of *P. aeruginosa* infection [255].

Patterns of various immunologic parameters and, in particular, isotypic antibody responses to *A. fumigatus* vary [11, 16, 18, 25, 34–36, 101, 256]. For example, elevated total serum IgE concentrations, presence of precipitins to *A. fumigatus*, and elevated serum IgE and/or IgG to *A. fumigatus* consistent with

ABPA occurs [16]. However, some patients with CF have exceedingly low total serum IgE concentrations, such as 100–200 ng/mL, but markedly increased levels of isotypic antibody to *A. fumigatus* [16]. These patients do not meet criteria for ABPA, but some eventually convert to ABPA.

When in vitro basophil histamine release was determined for patients with CF with and without ABPA (all patients had sputum colonized with *A. fumigatus*), a trend toward greater histamine release was found in basophils from patients with ABPA [25]. This finding may have pathological importance if the data can be extrapolated to pulmonary mast cells.

Sensitization to *A. fumigatus*, defined as immediate cutaneous reactivity by epicutaneous testing to a mixture of *Aspergillus* antigens (ALK) or IgE antibody graded as 2+ in a CAP immunoassay (Pharmacia), was present in 31 (18%) of 173 patients with CF, among whom the mean age was 14 years [19]. The total serum IgE concentration was increased in *A. fumigatus*-sensitized patients (433 vs. 82 IU/mL, comparable to 1012 vs. 197 ng/mL) [19]. The presence of *Pseudomonas* species in sputum, but not sensitization to *A. fumigatus*, was associated with decreases in FEV₁. The *Pseudomonas*-colonized patients had a mean FEV₁ of 63%, compared with 90% for noncolonized patients [19]. The authors reported that sensitization to *A. fumigatus* and elevated total serum IgE concentrations were associated with declines in FEV₁ compared with that in patients without increased total serum IgE concentrations [19]. A limitation of this study was lack of screening for ABPA.

Analysis of serial responses should be considered in light of data from a series of 118 patients with CF, of whom 6 (5%) had concomitant ABPA [23]. When 97 patients without ABPA were evaluated over the course of a 12-year period, 31 were found to have lost serological evidence of serum IgE to *A. fumigatus*, and 29 had lost evidence of serum IgG to *A. fumigatus* [22]. Immediate cutaneous reactivity was lost in 8 patients [23].

There are many additional reports of further immune responses to *A. fumigatus*, cytokine production, T cell activation,

IgE production or down-regulation, and basophil release [4, 104, 105, 136, 137, 160, 241, 257–261].

Specific Tests and Markers for Diagnosis of ABPA in CF

The skin test has been demonstrated to be a good diagnostic tool [23, 262]. However, cutaneous skin reactions, immediate (type I) or late (type III), to *A. fumigatus* antigens have been found in 31% of patients with CF without ABPA [99]. Twenty percent of west European patients with asthma were found to have positive results of skin testing for *A. fumigatus* [135, 263]. In other studies, 31%–59% of sample populations of patients with CF were reported to have positive results of skin testing for *A. fumigatus* [17, 136]. For the wheal of immediate skin sensitivity, some authorities have suggested that a diameter of ≥ 4 mm be considered a positive result; others have suggested ≥ 3 mm, with surrounding erythema.

Precipitating antibodies have been found to be a sensitive marker, both in CF [11, 12, 23] and in asthma [264]. Although a key diagnostic feature of ABPA, precipitating antibodies have been found to be present in patients with CF without ABPA. Furthermore, the level of these antibodies fluctuates over time [23].

Total IgE has been reported to be a valuable measure for diagnosing ABPA in CF [11, 12, 23], as well as in asthma [15, 264], but the values vary with age. Furthermore, various cutoff levels have been suggested. Values of total IgE as high as >500 IU/mL [34] or even >1000 IU/mL have been suggested as diagnostic criteria [265].

The presence of distal airway obstruction is a useful diagnostic criterion, but it has a low specificity. A high percentage of patients with CF have this symptom because of their primary disease [266].

Eosinophil counts have been found to be of limited value in diagnosing ABPA in both CF [11] and asthma [246]. Furthermore, eosinophilia may be present because of chronic *P. aeruginosa* infection.

Lung infiltrates and bronchiectasis often seem to occur too late in the progression of the disease to be valuable in making the diagnosis, and, specifically in patients with CF, these findings are often present because of bacterial infections [11, 266]. Laufer et al. [11] found that chest radiographs of patients with CF revealed infiltrates and/or bronchiectasis in 80%, which make radiographic distinction between patients with CF and those with CF plus ABPA impossible. Limitations of CT scanning are discussed below (see Imaging of ABPA in Patients with CF).

The presence of specific anti-*A. fumigatus* IgE antibodies is a sensitive indicator for ABPA in CF [11, 12, 23] or in asthma [15, 39, 264].

Precipitating antibodies to *A. fumigatus* are generally of the IgG isotype, in particular of the IgG1, IgG2, and IgG4 subclasses

[267, 268]. Increased levels of specific IgA and IgM antibodies to *A. fumigatus* have been reported [269–272], but IgE and IgG are the isotypes most markedly elevated in patients with CF. A specific pattern of increased levels of total IgG and IgG1, IgG2, and IgG4 antibodies to *A. fumigatus* has been reported in patients with CF with ABPA [25]. Specific immunoglobulins might preferably be measured by means of a panel of recombinant *A. fumigatus* antigens in patients with asthma, as proposed by Cramer et al. [273].

Culture of sputum for *A. fumigatus* yielding positive results has been regarded as a sensitive marker for ABPA [12, 245], whereas others have considered it only a minor criterion [17, 31, 245]. IgE response to steroids has also been suggested as a criterion for diagnosis of ABPA [26, 32, 35, 265].

Criteria for Diagnosing ABPA in CF

Laufer et al. [11] stated in 1984 that skin tests with a validated antigen and measurement of total IgE and specific IgG antibodies are the best screening tests for ABPA in CF. However, in their study, precipitating antibodies were detected in $>50\%$ of their patients with CF ($n = 147$).

In a 12-year longitudinal study of 118 patients with CF, Hutcheson et al. [23] found that 42% of patients without ABPA ($n = 112$) had a positive result of skin testing to *A. fumigatus*, 42% were positive for precipitating antibodies, 54% were positive for IgE to *A. fumigatus*, and 23% had IgE levels of >500 IU/mL. According to these studies, the best screening tests remained specific skin testing and measurement of IgE and precipitating antibodies to *A. fumigatus* [23].

The diagnosis of ABPA in CF is difficult, and may often be delayed, because many of the diagnostic criteria overlap with common manifestations of CF. Unlike the case in asthma, pulmonary infiltrates, bronchiectasis, and obstructive lung disease are common manifestations of lung disease in patients with CF, with or without ABPA, resulting from recurrent and chronic bacterial infections. Atopy, as well as an onset of a variety of immune responses to *A. fumigatus* antigens early in life in patients with CF, complicates the interpretation of various serological parameters for the diagnosis of ABPA [18, 22, 40, 101]. Early diagnosis and treatment aimed at suppression of the inflammation are, however, important to prevent irreversible lung tissue damage [262].

The following minimal essential criteria for diagnosing ABPA in patients with CF have been proposed: (1) asthma or airflow obstruction; (2) immediate cutaneous reactivity to *Aspergillus* species; (3) elevated total serum IgE concentration (>417 IU/mL or >1000 ng/mL); (4) elevated serum IgE to *A. fumigatus* and IgG to *A. fumigatus*; and (5) central bronchiectasis. The use of classic criteria for ABPA has been proposed as well, including peripheral blood eosinophilia [24]. However, a diagnosis not requiring asthma was used in the Epidemiologic

Study of Cystic Fibrosis (ESCF) conducted in the United States and Canada [26]. The criteria proposed for diagnosis of ABPA in CF in the ESCF were as follows. Two of the following 3 criteria are required: (1) immediate cutaneous reactivity to *A. fumigatus*; (2) precipitating antibodies to *A. fumigatus*; and (3) total serum IgE of >1000 IU/mL. In addition, at least 2 of the following are required: (1) bronchoconstriction; (2) peripheral blood eosinophilia >1000 eosinophils/ μ L; (3) history of pulmonary infiltrates; (4) elevated serum anti-*A. fumigatus* IgE or IgG; (5) *A. fumigatus* in sputum found by smear or culture; and (6) response to steroids.

In a series of 14,210 patients with CF who were >4 years of age studied during 1993–1996, 281 (2%) received a diagnosis of ABPA [26]. Of the whole series of patients, the physical examination finding of wheezing had been reported in 11%, and 19% of patients were recognized as having asthma in the preceding 6 months [26]. Recovery of *Pseudomonas* species occurred in 62% of patients in the past year. Ten percent of patients had FEV₁ of >100% predicted. From the cohort of patients with a diagnosis of ABPA, wheezing had been documented in 17%; asthma was present in 30%, with recovery of *Pseudomonas* species from 73%. Also, 10% of patients had FEV₁ of >100%. The OR for ABPA if the FEV₁ was <70% was 2.0. *A. fumigatus* was recovered from sputum of 34% of patients with ABPA and 8% of patients with CF without ABPA [26].

Another proposed set of diagnostic criteria have been used by Skov and colleagues [25, 34], in which 3 key findings are necessary to diagnose ABPA in patients with CF: (1) positive results of culture of sputum for *A. fumigatus*; (2) precipitating antibodies to *A. fumigatus*; and (3) increased anti-*A. fumigatus* IgE by radioallergosorbent test (RAST) [24, 33].

In the European Epidemiologic Registry of Cystic Fibrosis (ERCF), consisting of 12,447 patients [265], 4 required diagnostic criteria were as follows: (1) immediate cutaneous reaction to *A. fumigatus*; (2) total serum IgE level of >1000 IU/mL; (3) multiple serum precipitins to *A. fumigatus*; and (4) physician suspicion of ABPA based on the presence of at least 1 of reversible bronchospasm or asthma, pulmonary infiltrates, peripheral eosinophilia (>1000 eosinophils/ μ L), *A. fumigatus* in sputum or hyphae on smear, or response to steroids.

With these criteria, the prevalence was reported as 7.8% (range, 2%–14%). It was demonstrated that patients with ABPA were more likely to be colonized with various pathogens, including *P. aeruginosa*, *Burkholderia cepacia*, and *Stenotrophomonas maltophilia*. Also, complications such as massive hemoptysis and pneumothorax were more likely to occur in patients with ABPA. However, the longitudinal decline in FEV₁ was not different in patients with ABPA and patients without [265]. There appear to be no other data with which to compare this finding. It is not clear which is the most useful parameter

to follow in patients with CF. The natural history of ABPA in CF remains poorly understood.

In light of various diagnostic criteria for ABPA in CF, a survey was conducted from 58 CF centers in the United Kingdom [274]. There was marked nonconformity with diagnostic and treatment approaches in 45 centers. Among criteria that “must be present,” *Aspergillus*-specific IgE was listed just 54% of the time, wheeze and/or cough 46% of the time, and total serum IgE of >1000 ng/mL 45% of the time [274]. These findings highlight the wide differences in reporting of the incidence of ABPA in the literature. With respect to the use of wheezing in diagnostic considerations, it must be noted that intercurrent viral infections can produce or increase wheezing.

Consensus Conference Proposed Diagnostic and Screening Criteria for ABPA in CF

After review of the literature, the Consensus Conference recommendations for diagnosis and for screening for ABPA in CF are as follows.

Classic case

1. Acute or subacute clinical deterioration (cough, wheeze, exercise intolerance, exercise-induced asthma, decline in pulmonary function, increased sputum) not attributable to another etiology
2. Serum total IgE concentration of >1000 IU/mL (2400 ng/mL), unless patient is receiving systemic corticosteroids (if so, retest when steroid treatment is discontinued)
3. Immediate cutaneous reactivity to *Aspergillus* (prick skin test wheal of >3 mm in diameter with surrounding erythema, while the patient is not being treated with systemic antihistamines) or in vitro presence of serum IgE antibody to *A. fumigatus*
4. Precipitating antibodies to *A. fumigatus* or serum IgG antibody to *A. fumigatus* by an in vitro test
5. New or recent abnormalities on chest radiography (infiltrates or mucus plugging) or chest CT (bronchiectasis) that have not cleared with antibiotics and standard physiotherapy.

Minimal diagnostic criteria

1. Acute or subacute clinical deterioration (cough, wheeze, exercise intolerance, exercise-induced asthma, change in pulmonary function, or increased sputum production) not attributable to another etiology.
2. Total serum IgE concentration of >500 IU/mL (1200 ng/mL). If ABPA is suspected and the total IgE level is 200–500 IU/mL, repeat testing in 1–3 months is recommended. If patient is taking steroids, repeat when steroid treatment is discontinued.
3. Immediate cutaneous reactivity to *Aspergillus* (prick skin test wheal of >3 mm in diameter with surrounding erythema, while the patient is not being treated with systemic antihista-

mines) or in vitro demonstration of IgE antibody to *A. fumigatus*.

4. One of the following: (a) precipitins to *A. fumigatus* or in vitro demonstration of IgG antibody to *A. fumigatus*; or (b) new or recent abnormalities on chest radiography (infiltrates or mucus plugging) or chest CT (bronchiectasis) that have not cleared with antibiotics and standard physiotherapy.

Consensus conference suggestions for screening for ABPA in CF

1. Maintain a high level of suspicion for ABPA in patients >6 years of age.

2. Determine the total serum IgE concentration annually. If the total serum IgE concentration is >500 IU/mL, determine immediate cutaneous reactivity to *A. fumigatus* or use an in vitro test for IgE antibody to *A. fumigatus*. If results are positive, consider diagnosis on the basis of minimal criteria.

3. If the total serum IgE concentration is 200–500 IU/mL, repeat the measurement if there is increased suspicion for ABPA, such as by a disease exacerbation, and perform further diagnostic tests (immediate skin test reactivity to *A. fumigatus*, in vitro test for IgE antibody to *A. fumigatus*, *A. fumigatus* precipitins, or serum IgG antibody to *A. fumigatus*, and chest radiography).

EPIDEMIOLOGY OF ABPA IN CF

Prevalence. As noted above, the ESCF reported that among 14,210 patients enrolled between 1993 and 1996, 281 cases (2%) met the ESCF criteria for ABPA [26]. For 1995, the Cystic Fibrosis Foundation (CFF) Registry reported an ABPA prevalence of 2.2% among patients with CF who were >5 years of age, very similar to the almost 2% for the ESCF [33]. The CFF registry does not require specific criteria for making the diagnosis. The ERCF reported data for 12,447 patients with CF from 224 CF centers in 9 European countries [265]. ERCF used diagnostic criteria of total IgE concentration of >1000 IU/mL with positive results of skin testing and presence of serum precipitins to *A. fumigatus*, together with additional clinical or laboratory parameters. The overall prevalence was 7.8%, with a range of 2% in Sweden to 14% in Belgium [265]. A study of 3089 Italian patients with CF revealed a prevalence of 6% [275]. The Italian survey used a combination of clinical and laboratory criteria for diagnosis.

Smaller single-center studies have had very variable prevalences ranging from 1% to 15% of patients. Different criteria were used in each study to define the diagnosis of ABPA, and there is not an agreed-upon level of total IgE necessary to make the diagnosis in patients with CF.

An ABPA study was conducted at the Verona CF Center (Verona, Italy) with the purpose of screening a large cohort of

patients with CF for ABPA [17]. Two hundred twenty-two consecutive patients with CF (approximately one-third of the patients with CF attending the center) between 6 and 36 years old were prospectively examined by use of a panel of clinical and immunologic investigations for ABPA (skin test with 12 common allergen extracts, including a mixture of *Aspergillus* antigens; additional intradermal skin testing with *Aspergillus* antigen; blood eosinophil count; measurement of total serum IgE; RAST for *A. fumigatus*-specific IgE; and detection of precipitins to *A. fumigatus* antigens). ABPA was considered probable if 5 of the 6 following criteria were present: (1) positive result of *Aspergillus* skin testing, (2) total IgE concentration >2 SD above the reference values for age, (3) positive results of RAST for *A. fumigatus*-specific IgE, (4) presence of *A. fumigatus* precipitins, (5) peripheral eosinophilia, and (6) evidence of asthma. ABPA was considered possible if only 4 criteria were present. Sensitization to *Aspergillus* was shown in 56% of patients. Twelve percent of patients had probable ABPA, and 11% had possible ABPA, without any substantial clinical differences (age, clinical score, radiographic score, or lung function) between them. Subjects with ABPA had worse clinical scores, radiograph scores, and results of pulmonary function testing than did those who were not sensitized to *Aspergillus* [17].

Acute ABPA was diagnosed in 16 patients (9%) followed at the CF Center at Stanford, and an additional 19 had total IgE concentrations of >500 IU/mL without meeting the 4 other criteria to make the diagnosis of ABPA [35]. Marchant et al. [32] reported a retrospective analysis of the case records of 160 children attending a tertiary-referral pediatric CF clinic in London. Sixteen children had a total IgE level of >500 IU/mL. Eleven (7%) of 160 had ≥ 6 other major criteria and were considered to have ABPA. Marchant et al. [32] suggested that a 4-fold rise in total IgE, particularly to >500 IU/mL, is strongly suggestive of the diagnosis of ABPA in children with CF. They believed that this elevation of IgE, together with positive results of testing for *Aspergillus* precipitins and in conjunction with clinical deterioration and new radiological shadowing, allowed simplification of the diagnosis of ABPA in CF.

Factors associated with ABPA. To summarize the ESCF data, there was increased prevalence of ABPA in males (59%), adolescents, and subjects with lower levels of lung function, wheeze, asthma, and positive results of culture for *Pseudomonas* [26]. Table 6 shows the age distribution of patients with a diagnosis of ABPA who were enrolled in the ESCF. Among children <5 years of age ($n = 3796$), there was only 1 reported case of ABPA.

In the ERCF study, there were no sex differences and a low prevalence among those <6 years of age [265]. After 6 years of age, the prevalence was almost constant at 10%. This population had lower FEV₁ than did those without ABPA, at any age. There was a higher rate of microbial colonization (*P. aeru-*

Table 6. Prevalence of allergic bronchopulmonary aspergillosis (ABPA) in cystic fibrosis by age.

| Age, years | No. (%) of patients with ABPA | Reported prevalence, % |
|------------|-------------------------------|------------------------|
| 5–10 | 62 (22) | 1.3 |
| 11–15 | 78 (28) | 2.4 |
| 16–20 | 65 (23) | 2.9 |
| 21–25 | 37 (13) | 2.3 |
| >25 | 39 (14) | 1.5 |
| Total | 281 (100) | |

NOTE. Data are from [26].

gimosa, *B. cepacia*, *S. maltophilia*, *Candida albicans*), pneumothorax, and massive hemoptysis, higher serum IgG levels, and poorer nutritional status in patients with ABPA. However, FEV₁ decline during the follow-up period was not substantially different in patients with ABPA and patients without for any subgroup based on age or disease severity at enrollment [265].

Regional distribution of ABPA prevalence in the United States in the ESCF varied from 0.9% in the southwest to 4.0% in the west (table 7) [26]. Whether these differences are true, meaning there is a regional difference, or whether the diagnosis was not pursued in the regions with lower prevalence is not known.

Staphylococcus aureus. Shah et al. [276] evaluated patients with bronchiectasis who did not have CF. They found that of those chronically infected with *S. aureus* (as evidenced by sputum cultures), there was a significant association with ABPA (OR, 8.8). In the ERCF study, patients with and without ABPA presented about the same rate of *S. aureus* colonization: 61% and 63%, respectively [265].

Role of atopy. The role of atopy was discussed above (see Immune Responses in CF Relevant to ABPA).

Aspergillus culture. Mroueh and Spock [31] reviewed the records of 236 patients followed at the Duke CF Center in Durham, North Carolina. Sixty (25%) had colonies of *A. fumigatus* in sputum and throat cultures. These patients were older and had more severe disease, as assessed by lower Shwachman-Kulczycki clinical-radiograph scores, than did patients who did not have evidence of *A. fumigatus*. In 15 of the patients with *A. fumigatus* (6.5% of the total population), the diagnosis was ABPA [31]. Nelson et al. [12] studied 46 patients with CF for colonization by and sensitization to *Aspergillus* organisms. The fungus was cultured from 21 (57%) of 37 patients who produced sputum. The incidence of ABPA during a 2-year period in that population of patients with CF was 11%.

The reported prevalence of *Aspergillus* species cultured from the sputum of patients with CF ranged from 1.3% [28] to 63% in patients being considered for lung transplantation [277]. In the ESCF, 34% of subjects with reported ABPA had respiratory

culture results positive for *A. fumigatus* versus only 8% of those who did not have ABPA [26]. In the ERCF data, *A. fumigatus* colonization occurred in 45% of patients with ABPA but only in 16% of patients without ABPA; in a study by Milla et al. [278] in Minnesota, positive culture results for *Aspergillus* did not seem to represent a special risk factor for ABPA. At Stanford University Medical Center (Stanford, CA), 31% of patients with CF with ABPA had positive results of sputum culture for *A. fumigatus*, and 100% had *P. aeruginosa* [35]. The association with *Pseudomonas* culture positivity has been noted above (see Diagnosis of ABPA in CF).

Sensitization to *A. fumigatus*. The range of reported sensitivity to *Aspergillus* is very variable, regardless of whether one looks at immediate hypersensitivity to skin testing, antibody by RAST test to *A. fumigatus*, serum precipitins, or IgG specific to *A. fumigatus*. The problem is compounded by the fact that the immune parameters change with time, they may diminish over time, and these changes may be independent of treatment. Most studies have shown that treatment with either corticosteroids or azoles is associated with a trend toward lowering total IgE levels and reducing evidence of sensitization to *A. fumigatus*.

Wojnarowski et al. [19] from Vienna studied the relationship between sensitization to *A. fumigatus* and pulmonary function tests in 118 patients with CF. Thirty-one children (26%) were sensitized to *A. fumigatus*. With adjustment for sex, age, height, and weight, sensitization was associated with lower values of FEV₁ and FEF_{25–75}. There was evidence for a more rapid decline in pulmonary function test results for *A. fumigatus*-sensitized patients with elevated total IgE levels than for those with normal IgE levels. Becker et al. [24] evaluated a population of 53 adult patients with CF and found that despite evidence of sensitization to *A. fumigatus* in 66% of patients, only 1 met their criteria for ABPA. Among 148 outpatients with CF aged 6–34 years, specific IgE to *A. fumigatus* antigens was present in 46% [279].

Natural history. ABPA appears to be associated with an increased decline in lung function [280], although this was not seen during the observation period of the ERCF study [265]. Nepomuceno et al. [35] reported that the decline of pulmonary function of patients with CF with ABPA was greater than expected during the 5-year course of their study (FEV₁, 3.3% annually). We need adequate longitudinal studies aimed at establishing the effect of ABPA on the decline in pulmonary function test results and, more generally, its influence on the final prognosis of CF.

In the 12-year longitudinal study of Hutcheson et al. [23], ABPA was diagnosed in 6 patients (5%). They also showed considerable variation over time in the levels of sensitization, as well as total IgE, the latter independent of steroid therapy. Ten percent of patients without ABPA had IgE levels of >1000

Table 7. Prevalence rates of allergic bronchopulmonary aspergillosis (ABPA) among patients with cystic fibrosis (CF), by North American geographic region.

| Geographic region | No. of patients with ABPA | Total no. of patients with CF | Reported prevalence, % |
|---|---------------------------|-------------------------------|------------------------|
| United States | | | |
| West | 69 | 1707 | 4.0 |
| Midwest | 58 | 1810 | 3.2 |
| South | 32 | 1384 | 2.3 |
| Southeast | 22 | 1186 | 1.9 |
| Mid-Atlantic | 21 | 1195 | 1.8 |
| Northeast | 28 | 1917 | 1.5 |
| Great Lakes | 22 | 2005 | 1.1 |
| Southwest | 17 | 1830 | 0.9 |
| All | 269 | 13,034 | 2.1 |
| Canada | 12 | 1176 | 1.0 |
| All in Epidemiologic Study of Cystic Fibrosis | 281 | 14,210 | 2.0 |

NOTE. Data are from [26].

IU/mL, some of whom were not classified as having ABPA because they had no symptoms, whereas others did not meet the criteria for ABPA. Of the 112 patients, 89 developed evidence of sensitivity to *Aspergillus*. In this study, which showed varying declines in parameters of *Aspergillus* sensitization, there was also considerable variation in the effect of steroids on these parameters.

Summary. There is no uniformity for making the diagnosis of ABPA in CF in the literature. Large-scale epidemiological studies are dependent, in their estimates of prevalence, on whether CF centers systematically screen for ABPA and have the appropriate clinical and laboratory facilities for testing. Two large-scale databases from North America show prevalence of ABPA in CF of 2% (ESCF) and 2.2% (CFF), whereas the large-scale registry from Europe (ERCF) shows a prevalence of 7.8%. Studies reported from single CF centers have prevalence figures from 1% to 15%. Factors associated with ABPA and CF include age (peaking in adolescence), atopy, severity of lung disease, and colonization with *Pseudomonas*. Respiratory cultures positive for *A. fumigatus* are common in CF, and the evidence thus far is limited to associating culture positivity in CF with ABPA.

RECOMBINANT ALLERGENS FOR THE DIAGNOSIS OF ABPA

The increased probability of developing IgE antibodies to allergens produced by *A. fumigatus* in asthmatics and patients with CF and the prevalence of positive skin reactions to *A. fumigatus* extracts in both groups of patients are well documented [18, 281]. The diagnosis of ABPA as a distinct clinical entity is complicated by a number of characteristics shared between ABPA and underlying pulmonary complications of CF

[281, 282]. Therefore, serological findings should strongly contribute to confirming or excluding ABPA that is suspected on clinical signs [282, 283], because the assessment of hypersensitivity to *A. fumigatus* is a prerequisite for the diagnosis of both fungal allergy and ABPA [11, 243]. ABPA, which for therapeutic reasons must be clearly distinguished from sensitization to *A. fumigatus*, should be ruled out in all patients at risk [243]. Standardized recombinant *A. fumigatus* allergens have the potential to substantially increase the specificity and sensitivity of diagnosis of *A. fumigatus*-related allergic diseases, including ABPA [8, 135, 136, 233].

The allergen repertoire of *A. fumigatus*. Western blot analyses of *A. fumigatus* extracts with sera of sensitized patients visualized at least 43 IgE-binding components [284]. However, sera from different patients recognize highly variable patterns of IgE-binding proteins depending on the extract used [285], sensitization status of the subject, and stage of the disease in patients with ABPA [152]. Although these studies clearly show the complexity of *A. fumigatus* as an allergenic source, they do not allow the biochemical identification of single allergens nor a diagnosis of sensitization resolved by component. A molecular biological approach based on phage-surface display technology and high-throughput screening of cDNA libraries enriched for IgE-binding clones [233, 235] allowed cloning and characterization of virtually all allergens produced by the fungus [286, 287]. To date, molecular characterization of 81 allergens from *A. fumigatus* by phage display, including all allergens approved by the International Union of Immunological Societies Allergen Nomenclature Committee, has been achieved [287]. The repertoire of structures detected covers secreted, cytoplasmic, and surface proteins with or without enzymatic activity, as well as structural and storage proteins, indicating that the combination

Table 8. Recombinant (r) *Aspergillus fumigatus* antigens evaluated in skin tests.

| Allergen | Specificity, n/N (%) ^a | Sensitivity, n/N (%) ^b | | Type of skin test | Reference(s) |
|----------|-----------------------------------|-----------------------------------|-------------------------------|-------------------|---------------------------|
| | | ABPA | <i>A. fumigatus</i> -allergic | | |
| rAsp f1 | 1/103 (99) | 53/66 (80) | 167/178 (94) | SPT, IDT | [228, 273, 289, 291, 292] |
| rAsp f3 | 0/20 (100) | 67/69 (97) | 35/48 (73) | IDT | [137, 233] |
| rAsp f4 | 0/5 (100) | 8/12 (67) | 0/12 | IDT | [233, 293] |
| rAsp f6 | 0/5 (100) | 5/12 (42) | 0/12 | IDT | [234, 293] |
| rAsp f8 | 0/6 (100) | 4/4 | ... | IDT | [294] |

NOTE. IDT, intradermal skin test; SPT, skin prick test.

^a No. of positive skin test results with allergen/no. of controls with negative skin test result with corresponding commercial extract.

^b No. of positive skin test results with allergen/no. of positive skin test results with conventional allergen extract.

of phage-surface display technology and robot-based screening is suitable for the rapid characterization of complete allergen repertoires produced by complex allergenic sources [288]. All allergens have been produced as recombinant proteins and tested for IgE binding in a pilot study with use of 10 sera from patients with ABPA. Although these preliminary results are not suitable for discrimination between major and minor allergens or for postulation of disease-specific allergens [135], they clearly demonstrate that all recombinant proteins are able to bind serum IgE of patients with ABPA.

Diagnostic value of recombinant *A. fumigatus* allergens.

The recombinant allergens Asp f1, Asp f2, Asp f3, Asp f4, and Asp f6 have been evaluated for their diagnostic performance in serological studies in asthmatic patients [8, 135, 232, 233, 273] and patients with CF [136, 233, 257] with or without ABPA. Additionally, Asp f1 has been evaluated in patients with atopic dermatitis and *A. fumigatus* sensitization [233, 289]. These studies showed that Asp f1 and Asp f3 are recognized by antibodies of *A. fumigatus*-sensitized persons with asthma with 100% specificity, reaching a sensitivity of 88% and positive and negative predictive values of 100% and 63% with respect to an overall diagnosis of sensitization to *A. fumigatus* [135]. Serological investigations involving Asp f4 and Asp f6 showed that allergen-specific IgE raised against these proteins could be detected exclusively in sera of patients with ABPA [135, 232, 233]. Eighty percent of the subjects with ABPA elicited an IgE response to Asp f4, 55% to Asp f6, and 90% to at least 1 of these allergens [135]. These results were confirmed by an additional study involving patients recruited in a different clinic [8]. Serological investigations of patients with CF yielded comparable results for the recombinant allergens tested [136, 233, 257]. The sensitivity of the diagnosis of *A. fumigatus* sensitization based on serology with Asp f1 and Asp f3 in patients with CF, compared with the Pharmacia-Upjohn CAP system as the reference standard, reached 98%, whereas the specificity,

defined by the lack of false-positive responses, reached 100%. Significant levels of allergen-specific IgE against Asp f4 and Asp f6 could be detected exclusively in sera of patients with CF who had ABPA. Ten (50%) of the patients with ABPA showed strong responses to both Asp f4 and Asp f6, 6 (30%) recognized only Asp f4, and 4 (20%) only Asp f6. The highly specific detection of ABPA on the basis of a positive response to Asp f4 and/or Asp f6 reached a sensitivity of 100%, with 100% specificity, according to the lack of false-positive responses. However, the final demonstration that a preparation acts as an allergen in vivo is its ability to elicit type 1 skin reactions in allergic persons and has to be demonstrated, especially for recombinant proteins, which may be incorrectly folded [290].

Skin tests with Asp f1, Asp f3, Asp f4, and Asp f6 on a limited number of patients corroborated the results obtained in vitro (table 8), confirming a high specificity of Asp f1 and Asp f3 for the detection of sensitization to *A. fumigatus* and of Asp f4 and Asp f6 for the detection of ABPA. In terms of diagnostic specificity, the recombinant *A. fumigatus* allergens have proven to be superior to allergen extracts, allowing a direct correlation between allergen-specific IgE in serum and wheal and flare reaction in skin tests [137, 292, 293]. Notably, a positive skin test response strictly correlated with the presence of allergen-specific serum IgE to the tested allergen in all cases [137, 292, 293], suggesting the possibility of relying on serological data to detect sensitization, thereby avoiding allergen challenges.

Conclusion. Recombinant *A. fumigatus* allergens can substantially improve the diagnosis of *A. fumigatus*-related allergic complications, including ABPA. In vitro and in vivo evaluation of a limited panel of recombinant allergens showed a high specificity for the detection of sensitization to *A. fumigatus*, as well as for the detection of ABPA. The sensitivity of diagnoses based on single recombinant allergens is, however, dependent on the incidence of sensitization to the allergen among the population studied. Therefore, to increase the diagnostic sen-

sitivity, a panel of recombinant allergens will be required. The results obtained with Asp f2, Asp f4, and Asp f6 in detecting allergen-specific IgE in sera from patients with a clinical diagnosis of ABPA suggest that these recombinant allergens represent potential standards to confirm the disease. However, multicenter studies involving large numbers of sera from patients with ABPA and from *A. fumigatus*-sensitized persons will be required to fully assess the diagnostic value of these new reagents. The availability of these allergens immobilized to the Pharmacia-Upjohn CAP system will allow a fully automated quantitative immunoassay determination of allergen-specific IgE and should facilitate such studies. Moreover, the availability of a large number of recombinant *A. fumigatus* allergens potentially covering the whole repertoire of IgE-binding proteins produced by *A. fumigatus* will allow us to reconstitute a complex synthetic extract by mixing single standardized components. This procedure overcomes problems related to the difficult standardization of *A. fumigatus* extracts and should contribute to a further improvement in accuracy in detecting sensitization to *A. fumigatus*.

IMAGING OF ABPA IN PATIENTS WITH CF

In this section, imaging findings seen in patients with CF with ABPA are described and compared with their appearance in patients with CF without ABPA.

Bronchiectasis

Ring shadows on posterior-anterior chest radiography may be a sign of bronchial wall thickening or of bronchiectasis. Central bronchiectasis is one of the hallmarks of ABPA [295], although the diagnosis can be made without bronchiectasis [10]. Central and peripheral (distal) bronchiectasis typifies CF. In a study comparing CT findings in asthmatic patients with and without ABPA, Ward et al. [296] found that bronchiectasis in ≥ 3 lobes is highly suggestive of ABPA.

Several reports have assessed the ability of CT to determine the etiology by the appearance and distribution of bronchiectasis [297, 298]. Cartier et al. [298] found, in both CF and ABPA in asthma, a pattern of bilateral, predominantly upper lobe bronchiectasis. Reiff et al. [297] included 30 patients with ABPA and 14 adults with mild CF in their study. Central bronchiectasis without peripheral bronchiectasis was seen in 11 patients with ABPA and in only 1 patient with CF. Santis et al. [299], however, in a study of adult patients with CF with mild disease, found central dilatation without peripheral bronchiectasis in 60% of 90 lobes with bronchiectasis. Reiff et al. [297] concluded that the 2 conditions could not be differentiated by the pattern of bronchiectasis.

The appearance of bronchiectasis has been divided into cylindrical, varicose (beaded), and cystic (saccular dilatation). Cy-

lindrical bronchiectasis has been reported as more typical of CF, whereas varicose and cystic bronchiectasis are more typical of ABPA [297, 300, 301]. Varicose and cystic bronchiectasis are not uncommon in CF, reported in 11% and 34% of bronchiectatic lobes [300].

Summary. Central predominance is common in ABPA without CF and rare in CF (literature consensus grade II-2). Distribution of bronchiectasis is similar in both diseases (grade II-2). Central varicose bronchiectasis suggests ABPA (grade III).

Pulmonary Infiltrates

The finding of fleeting pulmonary infiltrates has been reported as suggesting probable ABPA in patients with asthma [302]. Infiltrates are most commonly seen in the midlung zones [303], sometimes in upper lobes [304]. Varying pulmonary infiltrates are also common in CF [305]. Persistent parenchymal opacity has been reported in a child with CF and ABPA [306]. Several authors have suggested that the response of infiltrates to steroids on chest radiography may be a useful way to identify infiltrates due to ABPA [31, 262, 295]. This response is variable, however, with some patients showing neither partial nor complete clearing of infiltrates despite clinical improvement [31].

No studies have compared the chest radiographic appearance of infiltrates due to ABPA with that of infiltrates from other causes in patients with CF. No study has used CT to evaluate infiltrates of different causes in patients with CF.

Summary. Infiltrates are common in both ABPA and CF (literature consensus grade III). Infiltrates are more likely to partially or completely clear in response to steroids in ABPA or CF with ABPA than in CF without ABPA, although this response is variable (grade III).

Mucus Plugging

A finding that has been reported in ABPA but not in CF is the appearance of high-attenuation mucus plugs [307, 308]. In a report by Logan and Muller [308] of a series of 14 patients with ABPA, high-attenuation mucus plugs were seen in 4 patients. Mucoïd impaction with normal attenuation is a common feature of both ABPA and CF. Centrilobular nodules and mucoïd impaction have been identified as CT features suggesting ABPA in asthmatic patients [296]. Mucoïd impaction is seen on CT in $>80\%$ of adults with CF [309, 310]. Centrilobular nodules in CF usually indicate distal mucoïd impaction and are a common finding as well. In a small group of asymptomatic patients with CF, Shah et al. [310] found centrilobular nodules in 50%.

Summary. High-attenuation mucus plugs have been reported in ABPA and not in CF (literature consensus grade III). Mucus plugs are common in CF and in ABPA (grade II-2). Centrilobular nodules are seen in CF and in ABPA (grade II-3).

Pleural Thickening

Angus et al. [311] found pleural thickening on CT in 14 of 17 asthmatic patients with ABPA compared with 3 of 11 asthmatic patients without ABPA. Pleural thickening is also seen in CF. In a study of advanced CF, pleural thickening was found on CT in 15 of 21 patients [300].

Summary. Pleural thickening is seen in ABPA and in advanced CF (literature consensus grade III).

Choice of Imaging Modality

The imaging features described above are best identified with CT (literature consensus grade III). CT is far more sensitive than chest radiography for the detection of bronchiectasis [299–301]. Mucus plugging in large bronchi can be seen on plain radiographs but is more frequently identified by CT [309]. Centrilobular nodules and high-attenuation mucus plugs can be identified only by CT.

Both conventional and high-resolution CT have advantages in the evaluation of ABPA in asthma [295]. The optimal technique for evaluation of ABPA in CF has not been determined and will likely depend on the specific findings that are found to be most useful in identifying ABPA in patients with CF.

CT uses more radiation than plain radiography and is more expensive. Whether there are any advantages to CT compared with plain radiography in the evaluation of pulmonary infiltrates due to ABPA is not known.

Summary

Evaluation of ABPA in patients with CF is limited by similar imaging findings in the 2 diseases. Although the appearance of CF is rarely confused with ABPA [300], findings that are used to establish the diagnosis of ABPA in patients with asthma are common in all patients with CF. No studies have systematically evaluated the ability of CT findings to identify ABPA in patients with CF. An additional limitation of the literature is that the criteria for the diagnosis of ABPA vary and often are not well described.

Although findings of central cystic or varicose bronchiectasis are more common in ABPA, they are sufficiently common in CF to limit their value in identifying ABPA in CF. The only reported imaging finding seen in ABPA and not in CF is the presence of high-attenuation mucus plugs. This finding has not been studied in patients with CF and has been identified in <30% of asthmatic patients with ABPA.

PHARMACOLOGY AND TREATMENT OF ABPA IN CF PATIENTS

Therapy for ABPA involves prophylaxis against and treatment of acute exacerbations, as well as prevention of end-stage fibrotic disease [312]. There are 2 aspects of treatment of ABPA.

The first is the attenuation of the inflammation and immunologic activity, for which corticosteroids are the mainstay of therapy [313, 314]. The second is the attenuation of the antigen burden arising from fungal colonization of the bronchial tree [313].

Therapy for ABPA is problematic in patients with CF. First, several of the diagnostic criteria of ABPA overlap with common manifestations of CF [21]. Therefore, treatment trials must be rigorous in defining ABPA in patients with CF. Second, CF and ABPA cause many of the same clinical and physiological derangements (e.g., pulmonary infiltrates and airflow obstruction), so it is difficult to attribute them specifically to a single disease. In addition, systemic glucocorticosteroids, the cornerstone of treatment of ABPA, have toxicities that may be especially of concern in patients with CF who are already prone to the development of diabetes, osteopenia [315], and infections. Furthermore, growth retardation may occur in patients with CF who receive corticosteroids before maturity [316]. Therapy must also take into account that the pharmacology of many drugs is altered in CF.

Systemic (Oral) Corticosteroid Therapy

Pharmacology. The anti-inflammatory effects of systemic corticosteroids are thought to be effective in ABPA by inhibition of phospholipase A₂ activity, arachidonic acid metabolism, chemotaxis, cell adhesion, tissue infiltration of inflammatory cells, and production of IL-1 and TNF. This results in a decrease in serum IgE and eosinophilia, clearing of pulmonary infiltrates, and reduction of bronchospasm. Onset of effect (increased β -adrenergic receptor sensitivity) may occur within hours, but anti-inflammatory effects are observed after 6–12 h [317]. Two cases of ABPA have been reported [37, 318] that were refractory to enteric-coated prednisolone but responded to non-enteric-coated preparations. Prednisolone absorption takes place in the jejunum. It has been suggested that prednisolone, which is released from enteric-coated preparations at a pH of 6.8, may not be absorbed in the jejunums of patients with CF, because the pH there is often <5.0 for prolonged periods [319].

There is no inhibitory effect of corticosteroids on *Aspergillus*. However corticosteroids may indirectly affect *Aspergillus* in ABPA by decreasing the conditions favorable to growth and the immunologic response to *Aspergillus* antigens [262, 320].

Adverse effects commonly associated with corticosteroid use include gastric discomfort, mental changes, unmasking diabetes, cataracts, weight gain, osteoporosis, and decreased growth [31, 37, 321]. A decreased immune response may be responsible for disseminated disease in some case reports [322]. Gastric discomfort may be minimized by coadministering with food.

Treatment. The few studies of ABPA in CF have been with small numbers of patients. These studies have not been double-

blind or controlled, and the corticosteroid dose has varied. However, despite these methodological problems, these limited data suggest that corticosteroids are useful in the treatment of ABPA in CF (literature consensus grade II-3). Nepomuceno et al. [35] treated 14 patients with ABPA and CF with prednisone at an initial dose of 2 mg/kg/day for 1 week, followed by 1 mg/kg/day for 1 week, followed by alternate-day dosing that was gradually tapered over several months to the lowest dose producing no rebound in IgE, wheezing, eosinophilia, or new infiltrates. The rapidity and extent of steroid tapering was variable and individualized. Seven patients concomitantly began treatment with itraconazole, and an additional 6 patients eventually received this antifungal agent. Minimal data concerning outcome or final corticosteroid dose were reported, but corticosteroids were effective in lowering the serum IgE level to less than half of initial levels. Mroueh and Spock [31] treated 15 patients with CF with ABPA with prednisone, beginning at 2 mg/kg/day, that was “reduced progressively, as allowed by the clinical course.” The 15 patients received a total of 22 courses of corticosteroids ranging from 2 weeks to 38 months. All patients reported symptomatic improvement. Twenty (91%) of 22 treated exacerbations resulted in an improvement in spirometric findings, and 8 (67%) of 12 had an improvement in serum IgE levels. Chest radiographic findings were generally improved, and no patient had worsening of chest radiographic findings.

Corticosteroids have been demonstrated to have efficacy in other series of patients with ABPA and CF, but precise doses were not reported [32, 37, 153, 323–328]. Initial doses of 1–2 mg/kg/day have been recommended [37]. Steroids are commonly tapered on the basis of clinical response and tolerance, but no consistent pattern has been used in studies to date. Interestingly, despite these data, the ERCF survey of European CF centers showed that only 56% of patients with ABPA and CF received oral corticosteroids, compared with 15% of patients with CF without ABPA [265]. Inhaled corticosteroids were given to 75% of patients with CF and ABPA, compared with 39% of patients with CF without ABPA [265].

Some authors have suggested that serum IgE levels be followed regularly in patients with ABPA [10], because IgE levels correlate with disease activity, and that corticosteroid therapy should be instituted even for asymptomatic patients if the serum IgE level doubles from the baseline value [10]. The great majority of IgE is not directed against *Aspergillus* antigens but is nonspecific [329]. Although the serum IgE level remains part of a constellation of clinical parameters used to decide when corticosteroids should be given, it cannot be used in isolation to make that decision [31, 32, 329].

Itraconazole and Other Antifungals

Although corticosteroids are the mainstay of therapy for ABPA because they attenuate inflammatory and immunologic activity,

corticosteroids have no effect on the antigen burden arising from fungal colonization of the bronchial tree. Reducing the fungal burden in the respiratory tract might decrease antigenic stimulation, reduce inflammatory response, ameliorate symptoms, and possibly reduce the long-term risk of disease progression [249].

Several studies of antifungal agents, oral or inhaled, for patients with ABPA have recently been reviewed [330]. Antifungal therapy, especially with itraconazole, appeared to be efficacious and steroid-sparing. However, these studies had small numbers of patients and a small percentage of patients with CF. Few of the studies were placebo-controlled, blind, or randomized. Doses ranged considerably in those reports, but, in general, 200–400 mg of itraconazole was given daily for 1–2 weeks and then gradually tapered over several months.

Pharmacology. Itraconazole is an azole antifungal, and a major metabolite, hydroxyitraconazole, has similar antifungal activity [331, 332]. These can decrease airway colonization and thus decrease the response to *Aspergillus*. Because it is a poorly soluble weak base, dissolution of capsule content (and thus absorption) requires an acidic environment that commonly occurs with meals [333, 334]. Itraconazole in cyclodextrin oral solution does not require dissolution, so it is better absorbed when taken on an empty stomach [335]. Although serum levels are greater with the solution because of better bioavailability, use is limited by bad taste and other gastrointestinal intolerance. Itraconazole is highly lipophilic and thus distributes widely to tissues such as the respiratory tract [336–339]. It is 95% bound to albumin, so tissue penetration might be higher in a malnourished patient. Metabolism occurs via phase I (hydroxylation) and phase II pathways (via CYP3A4) in the liver. Itraconazole blood levels may take 1–2 weeks to reach steady state [333, 338]. Itraconazole inhibits CYP3A4 fungal cell wall ergosterol synthesis, and this is thought to be its primary mechanism of action [338]. Serum sampling (4 h after a dose after 1–2 weeks of therapy) is warranted in patients with life-threatening disease, those not responding to therapy, patients taking concomitant medications that lower acid production or are prone to cause other drug-drug interactions, or those with liver failure [340]. Measurement of concentrations in sputum may even be more directly relevant.

Several drug interactions may occur [341]. Therapy may be ineffective in patients given gastric acid-lowering therapies, such as histamine₂ blockers (e.g., ranitidine and famotidine), proton pump inhibitors (e.g., omeprazole and pantoprazole), antacids, or drugs that contain antacids (e.g., didanosine). To avoid this interaction and maximize absorption in such patients, itraconazole may be coadministered with 8 oz (236 mL) of a cola beverage [335, 339]. Orange juice may produce the same acidification [342], although grapefruit juice should be avoided because of its possible effects on gut CYP3A4 enzymes.

Itraconazole is not only a substrate for CYP3A4 but also an inhibitor. Therefore, medications metabolized by this pathway may reach toxic concentrations. Those historically associated with significant clinical effects, such as QT prolongation, include terfenadine and cisapride [343]. Oral midazolam is also significantly metabolized by gut and hepatic CYP3A4, and exaggerated effects have been noted after use of midazolam for as little as 4 days [344]. Methylprednisolone levels have also been increased because of this effect, but prednisone levels have not [249]. Elevated levels and toxicity from other medications metabolized by CYP3A4, such as cyclosporine [345], tacrolimus, and oral hypoglycemic agents, may occur when they are given to patients receiving itraconazole. Enzyme inducers, such as phenytoin, rifampin, and isoniazid, can significantly decrease itraconazole concentrations and effects [341, 346, 347].

Adverse effects include gastrointestinal upset, fever, rash, headaches, dizziness, fatigue, and decreased libido [348]. Evidence of hepatic toxicity (elevations in transaminase levels) is common, but it is often transient and self-limited. Much less common are liver failure and heart failure, so periodic monitoring of liver enzymes and symptomatic assessment of clinical signs and symptoms are warranted.

Treatment. Denning et al. [349] performed an open-label trial of itraconazole 200 mg twice daily in 6 patients with ABPA for 7 courses of therapy. Three of the patients had CF, and they represented 4 courses of therapy. These 4 patients all had an improvement in spirometric findings and a slight reduction in prednisone dose during therapy, although the prednisone dose at the termination of therapy was still 16–40 mg/day. Nepomuceno et al. [35] retrospectively examined 16 patients with ABPA and CF, among whom 13 patients eventually received itraconazole. These authors compared days during itraconazole treatment with days when itraconazole was not being taken and found that the daily prednisone dose was lower (14.2 ± 3.6 vs. 26.7 ± 6.5 mg; $P = .05$) and the number of acute episodes over the course of the 5-year study period was less (0.5 ± 0.3 vs. 1.1 ± 0.2 ; $P = .0009$) when subjects received itraconazole.

Recently, a randomized, double-blind, placebo-controlled trial of oral itraconazole at 200 mg twice daily for 16 weeks in treating 55 patients with ABPA was conducted [249]. The overall response, assessed by a combination of improvement in pulmonary symptoms, pulmonary function, chest radiographic results, and serum IgE levels, was superior in the itraconazole group compared with those receiving placebo (13 of 28 responders [46.4%] vs. 5 of 27 [18.5%]; $P = .04$). Benefits continued and were realized in some initial nonresponders in a follow-up open-label study phase [249]. However, none of the patients enrolled in this study had CF. This study suggested that an itraconazole-susceptible isolate may be required for response, thus suggesting the utility of performing in vitro susceptibility testing of isolates from the patient's sputum.

On the basis of these data, there is limited evidence that itraconazole is useful in patients with CF with exacerbations of ABPA (literature consensus grade III) and that it may facilitate a decrease in corticosteroid use (grade III). There is insufficient evidence to recommend other oral or inhaled antifungal agents. The role of itraconazole and other antifungal therapy in preventing pulmonary fibrosis and chronic pulmonary dysfunction from ABPA in patients with CF has not been explored, and such therapy cannot be recommended.

Inhaled Corticosteroids

Although several small case series have suggested that inhaled corticosteroids are useful in treating patients with ABPA without CF [350–353], a double-blind, multicenter study conducted in the United Kingdom in the 1970s of beclomethasone, at 400 μ g/day without a spacer (a volume holding chamber used with steroid metered-dose inhalers), failed to demonstrate clinical benefit [354]. Inhaled corticosteroids have been shown minimally to reduce bronchial hyperresponsiveness in patients with ABPA without CF [355]. Nepomuceno et al. [35] treated 2 of 16 patients with ABPA and CF initially with inhaled high-dose fluticasone. One of the 2 also received itraconazole. In both patients, clinical and serological courses were “favorable,” and oral prednisone therapy was not required during follow-up. Given this extremely limited information, inhaled corticosteroids cannot be recommended for initial therapy or for prevention of pulmonary fibrosis and chronic pulmonary dysfunction from ABPA in patients with CF.

Other Anti-Asthma Drugs

No clinical trials have been conducted to determine the optimal bronchodilator regimen for patients with ABPA with or without CF. Montelukast sodium, a leukotriene antagonist, was shown to increase exercise tolerance and peak flow rate in an open-label study of 11 patients with CF [356]. Interestingly, the patients who benefited most had serological results positive for *Aspergillus*. At present, although not supported by any clinical trials, a rational approach would be to use inhaled corticosteroids and leukotriene modifiers for the asthma component of ABPA, as recommended by National Institutes of Health asthma guidelines (literature consensus grade III) [357].

Treatment Guidelines

There are minimal data to formulate conclusive treatment recommendations for ABPA in CF. This section should be viewed not as a definite statement concerning treatment but rather as a guideline. The evidence to support these recommendations is based largely on uncontrolled case series and opinions of experts in the field (literature consensus grade III). The clinician should be aware that pulmonary exacerbations in a patient with ABPA and CF could be related to ABPA, pulmonary infection,

Table 9. Treatment recommendations for allergic bronchopulmonary aspergillosis (ABPA) in cystic fibrosis (CF).

| Total serum IgE, IU/mL | Pulmonary symptoms and/or worsening PFT results | New infiltrates on CR or CT | Positive serology ^a | Treatment recommendation(s) |
|---|---|-----------------------------|--------------------------------|--|
| >1000 or >2-fold rise from baseline | Yes | Yes | Yes | Treat for ABPA |
| >1000 or >2-fold rise from baseline | No | No | Yes | No treatment; monitor IgE, CR, PFT |
| >1000 or >2-fold rise from baseline | No | Yes | Yes | Treat for CF-related infection; consider treatment for ABPA if no response |
| >1000 or >2-fold rise from baseline | Yes | No | Yes | Consider treatment for ABPA, CF-related infection, and/or asthma |
| >500 in the past; no change from baseline | Yes | Yes | Yes | Treat for CF-related infection; consider treatment for ABPA or asthma if no response |
| 500–1000 | Yes | Yes | Yes | Treat for ABPA |

NOTE. CR, chest radiography; PFT, pulmonary function testing.

^a Aspergillus-specific IgG or IgE or presence of precipitins to *Aspergillus fumigatus*. Because these test results may not be available quickly, they are not required for initiation of therapy but should be obtained.

asthma, or a combination of these entities. The clinician should carefully monitor the response to therapy to confirm the suspected cause of the pulmonary exacerbation and be vigilant about additional and alternative causes for the pulmonary decompensation.

These treatment guidelines are divided into 2 parts. First, a decision has to be made about whether a patient with ABPA and CF should be treated for an exacerbation of ABPA. At times, this decision is clear, because all of the clinical features of the illness are typical of an ABPA exacerbation. At other times, this decision is difficult, because the clinical presentation displays features of ABPA, a CF-related infection, and asthma. The first part of these guidelines outlines recommendations for when therapy for ABPA should be given to a patient with CF. Treatment recommendations are proposed for scenarios of different clinical presentations. The second part of these guidelines recommends specific pharmacotherapy for an ABPA exacerbation in CF.

When Should a Patient with CF be Treated for ABPA?

Treatment Scenarios

Table 9 summarizes treatment recommendations for various scenarios of ABPA and CF.

Scenario 1: Definite exacerbation of ABPA.

- Positive results of serology for ABPA (*Aspergillus* precipitins or *Aspergillus*-specific IgG or IgE)
- Total serum IgE of >1000 IU/mL or a >2-fold rise from baseline
- New infiltrates on chest radiography or chest CT
- Worsening pulmonary function and/or pulmonary symptoms.

Recommendation: Treat for ABPA.

Comment. The clinician must be cognizant that a con-

comitant additional pulmonary process may be present, such as a CF-related pulmonary infection. If clinical features, such as purulent sputum and leukocytosis with “left shift” (i.e., an increased percentage of immature forms) in the absence of systemic steroid therapy are present, the clinician should consider the concomitant use of antibiotics. The presence of cavitation on chest radiography might suggest a staphylococcal infection, mycobacterial infection, or the presence of invasive fungal disease. Because results of serology for specific *Aspergillus* antibodies may be delayed, the clinician may treat for an ABPA exacerbation without these data but should ensure that these serological studies are obtained and consider alternate causes of pulmonary decompensation (e.g., asthma) if they yield negative results.

Scenario 2: Asymptomatic ABPA-S

- Positive results of serology for ABPA (*Aspergillus* precipitins or *Aspergillus*-specific IgG or IgE)
- Total serum IgE of >1000 IU/mL or a >2-fold rise from baseline
- No new infiltrates on chest radiography or chest CT
- Stable pulmonary function and/or pulmonary symptoms.

Recommendation: No therapy, monitor for ABPA.

Comment. These patients do not require therapy but are at risk for development of an ABPA exacerbation. Monitoring should include periodic measurement of serum IgE levels, *Aspergillus*-specific serology, spirometry, and chest radiography or CT.

Scenario 3: ABPA-S, worsening radiographic results, no symptoms

- Positive results of serology for ABPA (*Aspergillus* precipitins or *Aspergillus*-specific IgG or IgE)
- Total serum IgE of >1000 IU/mL or a >2-fold rise from baseline

- New infiltrates on chest radiography or chest CT
- Stable pulmonary function and/or pulmonary symptoms.

Recommendation: Treat for CF-related bacterial infection. If no improvement, consider a trial of therapy for ABPA.

Comment. Although such patients may have exacerbations of ABPA, therapy involves high doses of corticosteroids. It is reasonable to treat such patients initially with a course of antibiotics for a CF-related pulmonary infection. Additional causes of pulmonary infiltrates should be sought as dictated by the clinical presentation. If there is a high index of suspicion for an exacerbation of ABPA (e.g., an extremely high serum IgE level or extreme increase in the serum IgE level), then therapy for ABPA may be started alone or concomitantly with therapy for CF-related infection.

Scenario 4: ABPA-S, pulmonary decompensation, no radiographic change

- Positive results of serology for ABPA (*Aspergillus* precipitins or *Aspergillus*-specific IgG or IgE)
- Total serum IgE of >1000 IU/mL or a >2-fold rise from baseline
- No infiltrates on chest radiography or chest CT
- Worsening pulmonary function and/or pulmonary symptoms.

Recommendation: Consider therapy for CF-related infection, ABPA, and/or asthma.

Comment. Clinical judgment must be used in this situation, because all 3 of the above pulmonary conditions have a reasonable likelihood of being present. If clinical features, such as purulent sputum or leukocytosis with “left shift,” are present, the clinician might favor use of antibiotics. If there is a high index of suspicion for an exacerbation of ABPA (e.g., an extremely high serum IgE level or extreme increase in the serum IgE level), then therapy for ABPA may be started alone or concomitantly with therapy for CF-related infection. It should be remembered that, because treatment of ABPA requires relatively high-dose corticosteroids, it may be prudent to treat for infection in equivocal cases and assess response to this therapy before initiating therapy for ABPA.

Scenario 5: Previous history of ABPA, pulmonary decompensation, radiographic change, no change in serum IgE

- History of ABPA-S
- Total serum IgE unchanged from baseline
- No new infiltrates on chest radiography or chest CT
- Worsening pulmonary function and/or pulmonary symptoms.

Recommendation: Consider therapy for CF-related infection. Consider therapy for relapse of ABPA if there is no response. Also consider treatment of asthma.

Comment. Unless the clinical presentation suggests many

features of asthma (e.g., wheezing), it is reasonable to initiate treatment for a CF-related infection. If the patient fails to respond, treatment for asthma or relapse of ABPA should be considered.

Scenario 6: Failure of therapy for CF, pulmonary decompensation, radiographic change, IgE of 500–1000 IU/mL

- Positive results of serology for ABPA (*Aspergillus* precipitins or *Aspergillus*-specific IgG or IgE)
- Total serum IgE of 500–1000 IU/mL
- New infiltrates on chest radiography or chest CT
- Worsening pulmonary function and/or pulmonary symptoms.

Recommendation: Treat for ABPA.

Comment. This is most probably an exacerbation of ABPA, and ABPA treatment should usually be instituted. If the clinical presentation suggests a CF-related infection (e.g., purulent sputum or leukocytosis with “left shift”), concomitant antibiotics could be considered, or antibiotics alone could be given, with treatment of ABPA reserved for failure of a course of antibiotic therapy.

Pharmacotherapy for ABPA in CF

This section outlines recommendations for the treatment of ABPA. The clinician must be aware that these recommendations should be viewed as a guideline, and actual treatment must be individualized on the basis of clinical presentation, response to therapy, and development of side effects from medications. Table 10 summarizes treatment recommendations for an exacerbation of ABPA in CF.

Corticosteroids. Corticosteroids should be used for all exacerbations of ABPA in CF unless there is a contraindication to their use (literature consensus grade II-3). Initial therapy: 0.5–2.0 mg/kg/day prednisone equivalent up to a maximum of 60 mg for 1–2 weeks, then convert to 0.5–2.0 mg/kg/day prednisone equivalent every other day for 1–2 weeks, then taper on the basis of IgE, chest radiography, spirometry, and pulmonary symptoms. A clinical response confirms the diagnosis of an ABPA exacerbation. An attempt should be made to begin to taper off corticosteroids in 2–3 months. If there is no response to therapy, the clinician should search diligently for other causes of pulmonary decompensation. If the patient relapses during the corticosteroid taper, corticosteroid dosages should be increased, itraconazole should be added, and a corticosteroid taper should be attempted if and when clinical parameters improve. A small percentage of patients may require chronic corticosteroid therapy.

Itraconazole. There are insufficient data to recommend itraconazole for initial therapy of an ABPA exacerbation in CF. Itraconazole should be added to therapy if there is a slow or poor response to corticosteroids, for relapse of ABPA, in cor-

Table 10. Pharmacotherapy for an exacerbation of allergic bronchopulmonary aspergillosis (ABPA) in cystic fibrosis (CF).

| Treatment, factor | Explanation |
|--|---|
| Corticosteroids | |
| Indications | All patients except those with steroid toxicity (grade II-3) |
| Initial | 0.5–2.0 mg/kg/day po prednisone equivalent, maximum 60 mg/day, for 1–2 weeks |
| Begin taper | 0.5–2 mg/kg/day every other day for 1–2 weeks |
| Taper off | Attempt to taper off within 2–3 months |
| Relapse | Increase corticosteroids, add itraconazole, taper corticosteroids when clinical parameters improve |
| Itraconazole | |
| Indications | Slow or poor response to corticosteroids, relapse, corticosteroid-dependent, or corticosteroid toxicity (grade III) |
| Dosing | 5 mg/kg/day, maximum dose 400 mg/day po unless itraconazole levels determined; b.i.d. dosing required when daily dose exceeds 200 mg |
| Duration | 3–6 months |
| Monitor | Liver function tests for all cases; itraconazole serum concentrations if concern of adequate absorption, lack of response, possible drug-drug interaction; serum concentrations of concomitant drugs with potential for drug-drug interaction |
| Adjunctive therapy | |
| Inhaled corticosteroids, bronchodilators, other antiasthma drugs | No evidence for use in ABPA; may be used for the asthma component of ABPA (grade III) |
| Environmental manipulation | Attempt to search for and modify mold spore exposure in refractory cases (grade III) |

NOTE. Grades refer to literature consensus grade (see table 5).

ticosteroid-dependent ABPA, and in cases of corticosteroid toxicity (literature consensus grade III). The initial dose should be 5 mg/kg/day, which may be given once daily unless the dose exceeds 200 mg/day, in which case it should be given twice daily. The daily dose should not exceed 400 mg/day unless low serum itraconazole levels are obtained (<1.5 to 2.5 $\mu\text{g/mL}$ by bioassay, which measures itraconazole and hydroxyitraconazole activity; high-performance liquid chromatographic assays, which measure itraconazole only, report concentrations severalfold lower) [339]. This is a situation in which use of the cyclodextrin solution might be considered before raising the dose. The duration of therapy should be 3–6 months. It is important to assess the clinical response after itraconazole withdrawal to assess whether it is still beneficial (e.g., prevents relapse and is corticosteroid-sparing). For patients receiving itraconazole, liver function tests should be obtained before therapy and should be repeated whenever there is any suspicion of liver dysfunction. Routine liver function testing after 1 month and then every 3–6 months if therapy continues should be considered. Concomitant medications should be meticulously reviewed to avoid a drug-drug interaction (see Itraconazole and Other Antifungals, Pharmacology section), and doses of concomitant medications and itraconazole should be adjusted accordingly. This may require determining serum concentrations of concomitant drugs and/or serum itraconazole concentrations. Determination of itraconazole concentrations should also be considered when there is a lack of clinical response or if there is concern about adequate drug absorption or patient compliance. Blood should be drawn 4 h after a dose; at steady

state, achieved during the second week of therapy, random samples may be useful.

Newer antifungals with anti-*A. fumigatus* activity [358] may offer similar benefits and need to be studied in a fashion similar to that for itraconazole [249].

Adjunctive Therapy

There is insufficient evidence to recommend inhaled corticosteroids, bronchodilators, or other asthma therapy for the treatment of ABPA per se, but it is reasonable to use these agents for the asthmatic component of ABPA as recommended by National Institutes of Health guidelines (literature consensus grade III) [357]. In refractory cases, it may be worthwhile to examine the patient's environment in search of significant mold exposure that can be modified, although this is not a proven prophylactic maneuver.

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Consensus Methodology

Drafts for development were circulated prior to the conference, updated versions were presented at the conference, and semifinal versions were completed in small group sessions.

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